**Role of the pheromone for orientation in the group foraging ant, *Veromessor*
*pergandei***

Cody A Freas, Marcia L Spetch

Department of Psychology, University of Alberta, Alberta Canada

Address for correspondence:

Cody A Freas

Department of Psychology

University of Alberta

Edmonton, Alberta T6G 2R3 Canada

Email: freascody@gmail.com

ORCID IDs

Cody A Freas: 0000-0001-7026-1255

Marcia L Spetch: 0000-0002-0472-073X

Abstract

Navigation is comprised of a variety of strategies which rely on multiple external cues to shape a navigator's behavioral output. An additional navigational challenge is coping with forces such as wind and water currents that push navigators off-course. Here, we explore the cue interactions that dictate orientation and foragers' ability to counter course altering rotational changes in the desert ant, *Veromessor pergandei*. We found a cross sensory interaction between the pheromone cue and the path integrator underlies correct orientation during the inbound journey. The celestial compass provides directional information while the presence of the trail pheromone acts as a critical context cue, triggering distinct behavioral responses (vector orientation, search and backtracking). A particularly interesting interaction occurs between the pheromone and the forager's vector state. While exposed to the pheromone, foragers orient to the vector direction regardless of vector state, while in the pheromone's absence the current vector triggers the switch between behaviors. Such interactions maximize the foragers' return to the nest and inhibit movement off the trail. Finally, our manipulations continuously pushed foragers away from their desired heading, yet foragers were highly proficient at counteracting these changes, steering to maintain a correct heading even at rotational speeds of $\sim 40^\circ/\text{s}$.

Keywords: Celestial Compass; Rotational Displacement; Path Integration; Multisensory; Cue integration

Introduction

Animals attend to a number of sensory inputs from their surroundings in order to guide their movement. Cue sets can range across multiple senses, including magnetic cues, the visual cues of the sky and surrounding landmarks, olfactory cues carried along air currents or deposited by conspecifics, as well as idiothetic cues such as the odometer portion of the path integrator. Navigators often employ multiple strategies either simultaneously or in separate segments of their journey and the triggering of distinct behaviors is controlled by these cue inputs (Cheng 2006; 2012). When all present cues agree, navigational performance often improves (Büehlmann et al. 2020ab; Czaczkes et al. 2011; Steck et al. 2011). However, if cue sets disagree, one will often dominate the navigator's behavior while the conflicting cue set is ignored (Freas and Cheng 2017; Gürter et al. 2011; Lebhardt et al. 2012;) or navigators will weight both and choose an intermediate heading (Freas et al. 2017; Lebhardt and Ronacher 2014; Legge et al. 2014; Narendra 2007). Cue set interactions can occur both within (Freas et al. 2019a; Lebhardt and Ronacher 2014) and across sensory modalities (Gürter et al. 2011; Lebhardt et al. 2012).

Path integration is a navigational system present across animal groups, (Collett and Collett 2000; Etienne and Jeffery 2004; Mittelstaedt and Mittelstaedt 1980; Wehner and Srinivasan 2003; Zeil 1998) and allows individuals to continuously update their current position relative to their starting point (Collett and Collett 2000; Wehner and Srinivasan 2003). In walking insects such as ants, this path integration derived vector involves the coupling of a celestial compass for directional information informed by multiple cues, including the sun's azimuthal position and the sky's polarization pattern (Wehner and Müller 2006) and a distance estimate dependent on a step counter or pedometer (Wittlinger et al. 2007).

When exploiting resource sites that contain many or replenishing resources, communicating information about the site to nestmates becomes advantageous (Jackson and Ratnieks 2006). In these environments, ant and bee species forage socially and communicate information that aids other nest/hive mates in finding food and returning home efficiently (Hölldobler and Wilson 1990; Lindauer and Kerr 1960; von Frisch 1967). In ants, social information in the foraging context is mainly available through chemical signals such as a pheromone trail network between goal locations, which can act both as a recruitment mechanism as well as help guide naive foragers to food sites (Czaczkes et al. 2015; Hölldobler and Wilson 1990; Hölldobler 1995). One recurring question is whether characteristics of the trail can

90 resolve the twofold ambiguity of its straight path by providing polarity information of the
91 inbound/outbound directions (Czaczkes et al. 2015). Ants using complex trail structures with
92 multiple branches or bifurcations (Acosta et al. 1993; Czaczkes et al. 2013b) use the geometry of
93 these structures to indicate the polarity of their trails (Jackson et al. 2004). However, conclusive
94 evidence of polarity in straight trails is lacking in ants (Czaczkes et al. 2015; Wetterer et al.
95 1992; Vilela et al. 1987).

96 In the current study, we explore the cross sensory cue interactions which drive orientation
97 behavior on the straight-line pheromone trails of the desert harvester ant, *Veromessor pergandei*,
98 as well as how these navigators cope with forces that push foragers off their chosen heading. *V.*
99 *pergandei* uses both individual and social cue sets in order to navigate in a fan and column
100 structure (Plowes et al. 2013, 2014). Foragers leave and return to the nest along a pheromone
101 marked column that extends between 3-40m. At the end of this column, termed the column head,
102 foragers fan out to collect food individually before re-entering the pheromone trail at the column
103 head. Foragers rely on a visual compass throughout their foraging journeys based on the celestial
104 compass portion of their path integrator and do not show evidence of attending to visual
105 landmark cues (Freas et al. 2019a,b; Freas et al. 2020; Plowes et al. 2019). Recent work has
106 highlighted the pheromone's role in navigation with the pheromone acting as a critical context
107 cue, dictating orientation changes as foragers re-enter the column by triggering the retrieval and
108 integration of local vector segments (Freas et al. 2020). Additionally, foragers have been shown
109 to use some unknown aspect of the column and their remaining path integrator state to dictate
110 when foragers begin to backtrack, a behavior meant to aid foragers that have overshot the nest
111 (Freas et al. 2019b).

112 Through three experiments manipulating the directional conflict between the inbound
113 pheromone direction and celestial compass, we show that: 1.) A multisensory interaction
114 between the pheromone and the path integrator underlies correct orientation on the column; 2.)
115 The pheromone's presence triggers foragers' continual orientation to the vector over other
116 behaviors (i.e. search and backtracking) as well as inhibits movement off the trail; 3.) This
117 pheromone/path integrator interaction occurs both with the forager's current vector as well as in
118 zero-vector foragers relying on a vector memory of the previous trip; 4.) Finally, foragers which
119 experienced rotational manipulations are adept at behaviorally counteracting these changes as
120 foragers remained correctly oriented to the fluid celestial compass direction.

General Methods

Study Site

Tests were conducted during November 2018 and March, April and October 2019 on two *Veromessor pergandei* nests. Nest 1 was located within the Verrado Trail System in Buckeye, Arizona, USA (33°29'50.13" N, 112°31'57.36" W). Nest 2 was located at the South Mountain Municipal Park in Phoenix, Arizona, USA (33°19'43.10" N, 112°01'02.60" W). *V. pergandei* is common within the Sonoran Desert, inhabiting visually rich environments consisting of scattered trees, cacti and brush. During testing, only foragers with a food piece were tested to ensure motivation to return to the nest.

Experiment 1. Two Column Displacement

Methods

On the afternoon of November 20th 2018, foragers at Nest 1 developed two directionally distinct pheromone column structures separated by 128° with the larger, more populated main column extending 7.1m while the second column extended 3.0m before foragers began to fan out foraging individually (Fig. 1A). Along the main foraging column, a 4 × 6 grid of 1m squares was constructed using string and metal pegs with the grid ending 20cm before the nest entrance (Fig. 1A). The width of the main column was ~60cm and was approximately 30cm on each side of the center gridline. In the first condition, foragers were collected from either the main column (4m) or the secondary column (at 3m) and displaced into the main column at the 3m release point (Fig. 1A). This release procedure resulted in a directional conflict for foragers collected at the secondary column between their current vector direction and the true nest direction which could be directed by multiple potential cue sets (main column pheromone cue, other social cues such as inbound/outbound nestmate communication and the surrounding terrestrial cues). After release, all foragers' paths were recorded using pencil and graph paper until they exited the testing area or 5min had elapsed.

Statistical Analysis

Forager paths were digitized using GraphClick (Arizona Software) by marking the path every 10cm and analyzed by calculating the foragers' heading directions and path distance at the onset of systematic search/grid exit. Pre-search paths (black paths; Fig. 1B,C) and the onset of

search was defined as the first sharp $\geq 60^\circ$ turn, followed by the forager continuing 20cm in the new direction to exclude detours around small obstructions. We also excluded turns within the first 50cm to allow foragers to choose a heading. For foragers that initiated search behavior within the 5-min test, the path was separated into the pre-search portion and search portion (grey paths; Fig. 1C). Directional measurements of forager paths were analyzed with circular statistics (Batschelet 1981; Zar 1998). Pre-search heading directions were analyzed using Rayleigh Tests to determine if data was non-uniform. If data was non-uniform, V-tests were used to determine if headings were grouped in a predicted direction (vector or true nest direction). Mean heading directions between groups was compared using a Watson-Williams F-test.

Results

Foragers collected on and released back onto the main column ($n = 10$) remained oriented with headings grouped in the true nest direction (Rayleigh Test; $Z = 9.93$; $P < 0.001$; V test at 0° ; $V = 0.996$; $P < 0.001$); these foragers all left the grid before the onset of search and entered the nest ($\emptyset = 0.4 \pm 3.6^\circ$; Fig. 1B). Foragers collected from the secondary column ($n = 12$) and released on the main column were also oriented (Rayleigh Test; $Z = 11.39$; $P < 0.001$), yet headings ($\emptyset = 136.4 \pm 8.4^\circ$) were not grouped in the true nest direction (V test at 0° ; $V = -0.707$; $P = 0.99$). Instead these headings were grouped in the vector direction (V test at 128° ; $V = 0.964$; $P < 0.001$). The mean headings directions at the onset of search were significantly different between the two conditions (Watson-Williams F-test; $F_{(1,20)} = 789.15$; $P < 0.001$). Secondary column foragers did not run off the full vector length, traveling only 57.7% (mean pre-search distance = $1.73\text{m} \pm 0.24$) of their 3m vector before search onset and none successfully returned to the nest (Fig. 1C). Results suggest the path integrator is dominant over other cue sets when orienting, though foragers exiting the pheromone trail did not maintain this heading for the full vector length.

Experiment 2a. Rotation Tests

Methods

On Column Testing

To further assess the interaction between the path integrator and the pheromone cue, we rotated the pheromone trail underneath inbound foragers using a $60\text{cm} \times 60\text{cm}$ wooden board. On its surface, a 4×4 grid of 15cm squares was drawn (Sharpie™). At both nests, at the onset of

column-forming activity in the morning or afternoon, this board was placed in the path of the forming column and ants were allowed to deposit a pheromone trail upon the board. To entice foragers to climb on to the board, a small ramp made of sand was formed on each end. Foragers were observed to travel freely along the board and deposit the trail pheromone during the column's initial formation and at no point were ants observed to form a trail around the board. At Nest 1, the board was placed 4.0m from the nest entrance on a 7.1m column (56% column distance) and at Nest 2 the board was placed 6.0m from the nest on a 12.0m column (50% column distance).

Inbound foragers with food were observed as they climbed onto the board and allowed to travel to the center (Fig. 2A). Once the forager neared the center, the board was carefully lifted ~10cm so as not to disturb the ramps and rotated 180° (mean rotational speed; Nest 1: $41.3^{\circ}/s \pm 1.3$; Nest 2: $40.4^{\circ}/s \pm 1.2$) in place randomly either clockwise or counter-clockwise (Fig. 2B). After rotation, the board was set back in place on the column and the forager was allowed to leave the board in its chosen direction (Fig. 2C). To exclude the possibility foragers were retreating due to being disturbed by the 180° manipulation, an additional condition was conducted at Nest 1 with the rotation increased to 360° ($48.6^{\circ}/s \pm 0.9$), 4m from the nest on a 6.7m column.

Distant Displacement Testing

Using Nest 1 (column length, 11.0m), testing was also conducted at a distant site where local cues would be uninformative (>50m from Nest 1). As with previous conditions, at the start of foraging activity a board was placed in the forming column (5.5m from the nest) for 45 min to allow the formation of a pheromone trail. This 'on-pheromone' board was then transported to the distant site. Here, it was leveled on the ground and oriented in the column to nest direction next to an identical 'off-pheromone' control board, that contained no deposited pheromone trail and was never used during on column testing.

Foragers were collected from the midpoint of the column (50% = 5.5m) using an opaque phial and transported to this distant site. Foragers were released on the on-pheromone board (50% Column On-Pheromone condition), 7.5cm from the board's edge in the column head compass direction and centered on the width of the board (30cm each side). Foragers were allowed to orient and travel to the center of the board. Once near the center, the board was lifted ~10cm and rotated 180° ($29.4^{\circ}/s \pm 0.7$). As with previous conditions, foragers' full paths were

recorded before, during and after rotation. Foragers that left the board before reaching the center were only included in the Pre-Rotation path analysis.

In the second condition (50% Column Off-Pheromone condition), foragers collected from the midpoint of the column length were released onto the off-pheromone control board. In initial testing, foragers failed to orient to the vector direction and travel to the center of the board when released 7.5cm from the edge, making rotational manipulation impossible. Given this lack of orientation, these foragers were released at the board center and we recorded forager paths until they left the board without rotation. Forager headings in this condition were calculated at 30cm from the release point.

As previous work (Plowes et al. 2019; Freas et al. 2019b) has hinted that foragers having run off part of their column vector may not orient to a vector without the pheromone's presence, we added a final distant displacement condition to the off-pheromone control board using full vector foragers collected from the column head (Column Head Off-Pheromone condition). These foragers were tested on the control board with a 180° rotation ($42.3^\circ/\text{s} \pm 1.5$) identical to the 50% On-Pheromone foragers.

Statistical Analysis

In all conditions, foragers' positions during Pre-Rotation, Rotation, and Post-Rotation phases were recorded using an HD camera (GoPro 5TM - 30fps, 3840 × 2160-pixel image size) positioned on a stationary tripod 1.2m above the forager facing down. Forager paths were digitized using GraphClick (Arizona Software) by marking each forager position during the Pre-Rotation, Rotation and Post-Rotation stages and were collected by marking the center of the individual's thorax at 10 frame intervals (0.33sec). Foragers' Pre-Rotation paths were collected starting as the forager climbed onto the board or at the phial release point and ceased at the start of the rotation. During the Rotation stage, in addition to the foragers' positions, body orientations were collected by marking where the first segment of the thorax met the base of the head and the petiole (Fig. 2B). Foragers' Post-Rotation paths started as rotational movement ceased and foragers' paths were collected until they left the board. Forager heading directions and body orientations were analyzed using Rayleigh Tests to determine if data was non-uniform. If data was non-uniform, V-tests were used to determine if headings were grouped in a predicted direction (vector or inbound pheromone direction).

In order to assess forager body orientation performance on a finer scale throughout the rotation, we separated this data for each forager into four quarters each representing the forager's body orientation during 25% of the rotation. For each forager, the angular error from the vector direction was determined by calculating the absolute difference between each foragers' mean body orientation within that quarter and the vector direction (at 0°). Angular error comparisons between quarters were compared using Repeated-Measures Friedman Tests with Nemenyi post-hoc comparisons.

Results

On Column

At Nest 1 and Nest 2 in both the Clockwise and Counter-Clockwise 180° rotation conditions, Pre-Rotation paths (Fig. 3A; Fig. S2A,E; light grey paths) were non-uniform and headings were grouped in the nest direction (Table S1; Fig. S1B,E; Fig. S2B,F). After the rotation, with the pheromone cue and the path integrator now in 180° conflict, forager path headings (Fig. 3A; Fig. S2A,E; dark grey paths) at both nests were still non-uniform and were now grouped only in the vector direction at 180° and not the inbound pheromone direction at 0° (Table S1; Fig. S1C,F; Fig. S2D,H).

During the rotation (Fig. 3A; Fig. S2A,E; black paths), forager mean body orientations remained grouped in the vector direction at both nests (Table 1; Fig. 3B; Fig. S2C,G). As might be expected given the manipulation was constantly rotating the individual's heading off course, these body orientations were typically biased in the direction of the rotation (to the right of the vector in the Clockwise condition and to the left of the vector in the Counter-Clockwise condition). At both Nest 1 and Nest 2 body orientations were biased in the direction of the rotation with Clockwise foragers' mean body orientation 17.31° and 3.43° to the right, while Counter-Clockwise foragers were 11.71° and 30.17° to the left of the vector direction respectively (Fig. 3B; Fig. S2C,G).

During Clockwise rotations at both Nest 1 and Nest 2, body orientation performance measured through angular error from the vector direction significantly changed throughout the rotation (Nest 1, Friedman Test; $\chi^2 = 13.5$; $P = 0.004$; Fig. S3A; Nest 2, Friedman Test; $\chi^2 = 10.39$; $P = 0.02$; Fig. S3C). Post-hoc comparisons revealed that angular error at Nest 1 during the 4th quarter of the manipulation was lower and significantly different compared to both the 2nd Quarter ($P = 0.01$) and the 3rd Quarter ($P = 0.01$), while at Nest 2 angular error during the 1st

Quarter was lower and significantly different compared to the 3rd Quarter ($P = 0.02$). However, these changes were not present during Counter-Clockwise conditions at both Nest 1 and Nest 2, as angular error did not significantly change through the rotation (Nest 1, Friedman Test; $\chi^2 = 2.46$; $P = 0.48$; Fig. S3B; Nest 2, Friedman Test; $\chi^2 = 6.60$; $P = 0.09$; Fig. S3D).

In both the Clockwise and Counter-Clockwise 360° conditions, Pre-Rotation paths (Fig. 3A, light grey paths) were non-uniform and headings were grouped in the nest direction (Table S1; Fig. S4B,E). After the 360° board rotation the directionality of both the vector direction and the pheromone cue were again in agreement (0°) and forager paths (Fig. 3A, Fig. S4A,E; dark grey paths) remained oriented and grouped in this direction (Table S1; Fig. S4C,F).

During the 360° rotation (Fig. 3A; Fig. S4A,D; black paths), mean body orientations remained well oriented and grouped in the vector direction in both conditions (Table S1; Fig. 3B). Mean body orientation was biased in the direction of the rotation for Clockwise foragers (13.12° to the right) but not for Counter-Clockwise foragers (4.72° to the right). Individual forager body orientation measured by angular error did not significantly change throughout the rotation during either Clockwise (Friedman Test; $\chi^2 = 1.26$; $P = 0.74$; Fig. S3E) or Counter-Clockwise rotations (Friedman Test; $\chi^2 = 4.92$; $P = 0.18$; Fig. S3F).

Distant Displacement Testing

Foragers collected at the mid-point of the column and displaced distantly expressed different orientation behaviors based on the presence of the pheromone. 50% Column On-Pheromone foragers exhibited Pre-Rotation paths (Fig. 4A; light grey paths) which were non-uniform with headings grouped in the vector direction in both the Clockwise and Counter-Clockwise conditions (Table S1; Fig. S5B,E). In contrast, when 50% foragers were distantly displaced to the control board (no pheromone), headings were uniformly distributed, suggesting the onset of search behavior (Table S1; Fig. S6A,B).

50% Column On-Pheromone foragers continued orienting to the vector after the board rotation with the vector and pheromone directions now in opposition (180° and 0° respectively). Post-Rotation paths (Fig. 4A, dark grey paths) were still non-uniformly-distributed and forager headings were now grouped only in the vector dictated direction (Table S1; Fig. 4A; Fig. S5C,F).

Column Head Off-Pheromone foragers (both Clockwise and Counter-Clockwise) exhibited Pre-Rotation paths (Fig. 4A, light grey paths) and Post-Rotation paths (Fig. 4A, dark grey paths) that were non-uniform and post-rotation headings were grouped in the vector

direction but not the pheromone dictated inbound direction when in conflict (Table S1; Fig. S7C,F).

During the rotation, foragers maintained mean body axes that were oriented and grouped in the vector direction (at 0°) in both the 50% Column On-pheromone and the Column Head Off-Pheromone conditions (Table 2; Fig. 4B). As in previous conditions, these body orientations were biased in the direction of the manipulation with Clockwise foragers' mean body orientation 2.66° and 35.58° to the right and Counter-Clockwise foragers 12.80° and 33.02° to the left of the vector direction respectively. Body orientation performance measured through angular error did not significantly change throughout the rotation in both 50% Column On-Pheromone foragers (Clockwise, Friedman Test; $\chi^2 = 0.75$; $P = 0.86$; Fig. S3G; Counter-Clockwise, Friedman Test; $\chi^2 = 4.09$; $P = 0.26$; Fig. S4H) and Column Head Off-Pheromone foragers (Clockwise, Friedman Test; $\chi^2 = 6.68$; $P = 0.08$; Fig. S4I; Counter-Clockwise, Friedman Test; $\chi^2 = 5.16$; $P = 0.16$; Fig. S3J).

Experiment 2b. Pheromone-Vector Interaction

Foragers in Experiment 2a, only oriented themselves in the vector dictated direction; however this direction always corresponded with the inbound/outbound pheromone column, whereas in Experiment 1 where following the vector direction led foragers off the pheromone trail, individuals began to search soon after. To further characterize the interaction between foragers' path integrator and the pheromone cue at the edges of the trail, in Experiment 2b we developed a set of rotation conditions to mirror this conflict.

Methods

Once again, the pheromone board was placed onto the forming column (column length, 6.1m) at one of two distances from the nest with the board's center at 3m, which was the approximate column midpoint, or 50cm which was the closest the board could be placed without disturbing the nest structure (board edge 20cm from nest entrance). Unlike previous conditions where the pheromone trail was allowed to form unrestricted in regards to column width, here foragers were only allowed to form the pheromone column along the center 30cm (width) of the rotation board (grey area Fig. 5A) with the outside 15cm bands restricted using 10cm plastic walls. These walls were present anytime foragers were not being tested, with the walls being removed just before an inbound test forager climbed onto the board and were replaced immediately after testing.

In the 3m Vector 90° condition, inbound foragers at the 3m distance were followed as they climbed onto the board and when the forager neared the center, the board was rotated 90° either clockwise or counter-clockwise ($30.9^\circ/\text{s} \pm 1.2$). This 90° procedure was repeated at the 50cm distance from the nest ($27.0^\circ/\text{s} \pm 0.6$). Observations during testing suggested 50cm foragers showed large amounts of uncertainty after the rotation, so we conducted a final control condition to test if this uncertainty was due to the small remaining vector. In this condition foragers were again tested at 50cm from the nest but the board was rotated 180° ($29.3^\circ/\text{s} \pm 0.5$).

Statistical Analysis

Foragers' positions during Pre-Rotation, Rotation, and Post-Rotation phases as well as their body orientation during rotation were recorded and analyzed identically to Experiment 2a. In addition to these analyses, body orientations and Post-Rotation headings were compared between conditions using multiple metrics.

For body orientations during rotation we compared both the circular variance using Var tests (Wystrach et al. 2014) and the magnitude of bias size away from the vector direction during rotation using Watson-Williams F-tests with Holm-Bonferroni corrections for multiple comparisons. Orientation bias size and variance were compared between the Clockwise and Counter-Clockwise tests in the same condition. For bias size, we first calculated the mirror of the orientations in the Counter-Clockwise data (Freas et al. 2017; Freas et al. 2019b). As there were no significant bias or variance differences ($P > 0.05$) between Clockwise and Counter-Clockwise data in all three conditions, they were combined for between condition comparisons.

Post-Rotation paths were compared in heading variance using the Var test and path straightness. Path straightness was analysed using the ratio between the straight-line distance from the forager's position when rotation stopped to the exit location with the forager's recorded path length. These path straightness values ranged between zero to one, with one being a straight path. Between condition path straightness comparisons were analysed with a Kruskal-Wallis rank sum tests with post hoc Dunn's comparisons adjusted using the Holm-Bonferroni method.

Results

In all three tests, Pre-Rotation headings (Fig. 5A; Fig. S10A,E, light grey paths) were oriented with headings grouped in the vector direction (Table S1) in both the Clockwise and Counter-Clockwise conditions (Fig. S8B,E; Fig. S9B,E; Fig. S10B,F).

After the 90° board rotation in the 3m vector condition, with the vector now directing foragers off the pheromone trail either 270° (Clockwise) or 90° (Counter-Clockwise), Post-Rotation forager headings were oriented with headings grouped in the vector direction and not the pheromone dictated direction in both the Clockwise and the Counter-Clockwise conditions (Table S1; Fig. S8C,F). Though a number of foragers hesitated/initially retreated when exiting the pheromone trail, 85% (Clockwise) and 90% (Counter-Clockwise) of foragers continued in this direction and left the board in the vector direction after exiting the pheromone trail (though even some of these foragers initially turned back near the pheromone trail edge; Fig. 5A; Fig. S8A,D). The remaining foragers hesitated at the edge of the pheromone trail and then retreated back into the pheromone trail and left the board in one of the pheromone directions (2 backward/1 forward Clockwise condition; 1 backward/1 forward Counter-Clockwise condition).

In the 50cm Vector 90° condition, Post-Rotation forager headings were uniform (Table S1; Fig. S9C,F). Foragers in this condition showed a high degree of uncertainty at the edge of the pheromone trail with a large number of foragers retreating back into the pheromone marked areas. Only 22% (Clockwise) and 21% (Counter-Clockwise) of foragers continued off the pheromone trail and exited the board in the vector direction. The remaining foragers retreated back into the pheromone trail and left the board in one of the dictated pheromone directions (7 backward/7 forward Clockwise condition; 7 backward/8 forward Counter-Clockwise condition). In contrast, 50cm Vector 180° foragers' Post-Rotation headings continued in the vector direction at 180° and not the pheromone dictated direction (Table S1; Fig. S10D,G). Unlike both 90° conditions, here all foragers exited the board in the vector direction while on the pheromone trail.

During the rotation, foragers in all conditions maintained mean body orientation, facing the vector direction at 0° in both conditions (Table 2; Fig. 5B; Fig. 10C,G). Consistent with previous conditions, body orientations in the 3m Vector 90° and 50cm Vector 90° conditions were biased in the direction of the manipulation with Clockwise foragers' mean body orientation to the right (11.03° and 2.45°) and Counter-Clockwise foragers to the left (9.57° and 28.00°) of the vector respectively. In the 50cm Vector 180° condition, Counter Clockwise foragers' mean body orientation was 18.25° to the left as predicted, but Clockwise foragers were also slightly biased to the left 1.76°.

In all three conditions, body orientation performance measured through angular error did not significantly change throughout the rotation during any Clockwise (3m Vector 90°, Friedman

Test; $\chi^2 = 1.20$; $P = 0.75$; 50cm Vector 90°, Friedman Test; $\chi^2 = 7.13$; $P = 0.07$; 50cm Vector 180°, Friedman Test; $\chi^2 = 5.78$; $P = 0.12$; Fig. S4K,M,O) or Counter-Clockwise condition (3m Vector 90°, Friedman Test; $\chi^2 = 5.84$; $P = 0.12$; 50cm Vector 90°, Friedman Test; $\chi^2 = 7.13$; $P = 0.07$; 50cm Vector 180°, Friedman Test; $\chi^2 = 5.63$; $P = 0.13$; Fig. S4L,N,P).

Between Condition Comparisons

During the rotation, mean body orientation showed no significant difference in variance (Var test; $P > 0.05$) or bias magnitude between the 3m Vector 90°, 50cm Vector 90°, and 50cm Vector 180° conditions (Watson-Williams F-test; $P > 0.05$).

Only after the rotation did we observe differences in forager behavior when comparing foragers' Post-Rotation heading directional variance and path straightness. Both heading variance and path straightness of foragers' Post-Rotation headings were significantly different between conditions (Heading variance, Var test; $V = 39.637$; $P < 0.001$; Path Straightness, Kruskal–Wallis test; $KW = 36.616$; $P < 0.001$). Heading variance decreased and was significantly different when the post rotation vector direction coincided with the continued presence of the pheromone in the 50cm Vector 180° compared to both 90° rotation conditions where the vector directed foragers off the pheromone trail edge (3m Vector 90°, Dunn's test; $P = 0.02$; 50cm Vector 90°, Dunn's test; $P < 0.001$). Additionally, vector length also appeared to play a role as foragers in the 50cm Vector 90° condition showed higher post rotation variance that was significantly different than the 3m Vector 90° condition (Dunn's test; $P < 0.001$). Path straightness did not differ between the 3m Vector 90° and 50cm Vector 180° conditions (Dunn's test; $P = 0.37$), but straightness was lower and significantly different for the 50cm Vector 90° condition compared to both the 3m Vector 90° condition (Dunn's test; $P < 0.001$) and 50cm Vector 90° condition (Dunn's test; $P < 0.001$).

Experiment 3. Vector Memory tests

In Experiments 1 & 2, foragers tested with a remaining vector continuously updated their orientation to the vector both during and after the rotation. However, previous work has shown that even zero-vector foragers orient in the vector direction when placed back into the column (Freas et al. 2019b). To further characterize how the pheromone interacts with vector state to influence forager behavior, we again created an artificial column at a distant location from the nest.

Methods

Here we used two identical 120cm × 60cm wooden boards to record paths along the trail for 60cm in each direction. One board was placed 1m (Column 8.3m) from the nest and foragers were allowed to create a foraging column along its surface for 45min, with plastic walls restricting movement to the centre 30cm (Fig. 6, grey area). Then this board was transferred to the distant site (>50m away) where it was leveled on the ground in the same compass orientation as on the column, next to an identical board with no pheromone. Three zero-vector conditions were conducted with returning foragers collected just before (< 10cm) entering the nest, where their current vector would be directionally uninformative. Zero-vector foragers were held in an opaque phial for 5min and then released either on the ‘on-pheromone’ or ‘off-pheromone’ boards (Zero-Vector On-Pheromone and Zero-Vector Off-Pheromone). For foragers on the on-pheromone board, the orientation of the board was randomly oriented with either the inbound or outbound pheromone direction in agreement with the column compass direction, resulting in half being tested as Zero-Vector 180° Rotated Pheromone foragers. As zero-vector forager paths were directionally distinct between on and off pheromone conditions we repeated this experiment with full vector foragers collected from the column head at 8.3m from the nest entrance (Column Head On-Pheromone, Column Head 180° Rotated Pheromone, Column Head Off-Pheromone).

Given the continued vector orientation in zero-vector foragers despite the current vector state, we added two final conditions. In the first, a Zero-Vector Sun Mirrored condition, we used the same procedure as the Zero-Vector On-Pheromone condition but here the location of the sun was mirrored 180° using a mirror on one side of the column and a board on the other side to block the sun’s natural location. In the Zero-Vector 90° Rotated Pheromone condition, the on-pheromone board was oriented to create a 90° conflict between the inbound column compass direction (0°) and the inbound pheromone direction (270°) so that maintaining the vector heading would require foragers to leave the pheromone trail.

Statistical Analysis

In all conditions, foragers’ full paths from release were recorded using an HD camera and foragers’ positions were collected every 10 frames (0.33s). Forager paths were analyzed by calculating both the forager’s initial heading direction at 30cm from the release point as well as their mean path heading. Mean path headings were calculated by compiling all foragers’

positions on the board relative to the release point. Forager heading directions were analyzed using circular statistics.

Results

In both the Zero-Vector On-Pheromone (Fig. 6A) and Zero-Vector 180° Rotated Pheromone (Fig. 6B) conditions, both foragers' headings at 30cm and mean path headings were non-uniform and headings were oriented and grouped in the nest compass direction regardless of pheromone orientation (Table S2). In contrast, Zero-Vector Off-Pheromone forager headings at 30cm and mean paths were still oriented but were not in the inbound compass direction (Table S2; Fig. 6C). Instead these forager headings were grouped in the opposite, nest-to-column-head compass direction, consistent with backtracking (heading at 30cm, V test; $V = 0.523$; $P = 0.003$; mean path, V test; $V = 0.742$; $P < 0.001$).

In all three Column Head conditions, both mean path headings and headings at 30cm were non-uniform and were grouped in the vector compass direction regardless of pheromone presence or orientation (Table S2; Fig. S11A-C). In both the Zero-Vector Sun Mirrored and Zero-Vector 90° Rotated Pheromone conditions, headings at 30cm and mean paths were uniform (Table S2; Fig. S11D,E).

Discussion

Trail Directionality

The pheromone cue alone shows no evidence of providing trail directionality, which is instead dictated by the celestial compass portion of the path integrator. When the pheromone trail was rotated by 180° foragers across all three experiments retained correct orientation to the vector direction despite the directional conflict with the inbound pheromone direction (Experiments 1-3) as well as in instances when the surrounding visual terrestrial cues and other social cues on the column also conflicted with the vector (Experiment 1). When tested either locally on the column or distantly on a pheromone marked board, foragers maintained correct orientation to the vector during rotation (Experiment 2). These directed headings persisted Post-Rotation, even when this forced foragers to travel outbound on the pheromone trail. When paired with the presence of the pheromone cue, vector-based orientation occurred regardless of vector state, with foragers ranging from full to zero-vector choosing to orient in the vector direction over the inbound pheromone direction. Notably, when the pheromone cue was absent, the remaining vector state

became a salient cue in determining orientation behavior, with full vector foragers remaining oriented to the vector, while foragers with half or no remaining vector displayed search or backtracking behaviors respectively.

Furthermore, when the vector and the pheromone were placed in 90° conflict, one might expect foragers that were hesitant to leave the pheromone trail (Experiments 2b & 3) to show some preference for the inbound pheromone direction if any directional information was present. However, even when foragers refuse to follow the vector off the pheromone trail, they still do not show any preference for the inbound pheromone direction. The complete lack of any orientation to the inbound pheromone cue in any condition unless it corresponded with the vector suggests that directional information is unlikely to be present, with the path integration system being the primary driver for inbound navigation. Instead, the pheromone cue appears to guide vector-based navigation with its presence/absence determining behavioral output.

Cue interaction – context cues trigger behavior

Navigating ants are known to attend to multiple cues and employ an array of strategies. The use of one navigational strategy over another or multiple strategies simultaneously is controlled by the current cue inputs (Cheng 2006; 2012). Typically, when cue integration is studied, both cue sets provide directional information. For example, many navigating ants attend to both their path integrator and learned cues for directional information (Wehner 2008; Cheng et al. 2009; Knaden and Graham 2016). Yet navigational behaviors can also rely on a directional cue's interaction with cues that are non-directional, such as the remaining vector distance of the path integrator, the remaining vector influencing when foragers begin to search and backtrack (Narendra 2007; Wystrach et al 2013; Freas et al. 2019b). Certain cues only become salient when other cues align. The desert ant *Cataglyphis fortis* will only orient to a CO₂ plume marking the nest entrance, when it is associated with a near zero-vector state (Büehlmann et al. 2012). Here, the celestial compass portion of the path integrator acts as the primary directional cue, however cue interactions between the pheromone and the vector dictate forager orientation, with the presence of the pheromone enhancing vector orientation over other navigational behaviors. Additionally, the pheromone cue also mediates the use of the pedometer portion of the path integrator, as a forager's remaining vector distance only triggered behavior changes in the absence of the pheromone.

When foragers were collected from the column head (full vector) and displaced distantly either with or without the presence of the pheromone, foragers successfully oriented to the vector direction. In contrast, foragers with half of their column vector run off and released onto ground with no pheromone did not orient to the vector direction and instead engaged in search behavior. Foragers collected from the same midpoint and displaced onto the pheromone board successfully oriented to the vector direction and maintained this direction during and after rotational manipulation. Foragers with the same vector state exhibited two distinct behavioral outputs based on the presence/absence of the pheromone cue. These results suggest that the presence of the pheromone cue triggers continual orientation to the vector direction regardless of the remaining vector distance estimate, leading foragers to continue to follow this cue even when the remaining vector is small (or zero, discussed below). Yet, when the pheromone cue is absent, a forager's remaining vector distance becomes salient in determining behavior, and triggers the onset of search well before the vector reaches zero.

Cue interaction – zero-vector foragers

Path integration is typically studied as working memory that is used during the current foraging trip and reset once the forager enters the nest (Knaden and Wehner 2006), yet foragers also retain long-term vector memories for subsequent foraging trips (Collett et al. 1999; Collett and Collett 2000; Ziegler and Wehner 1997). In the current study, interactions between the pheromone and path integrator even occur in zero-vector foragers. In the presence of the pheromone cue, foragers continued orienting in their recently run off vector direction regardless of the underlying direction of the pheromone. Yet, the presence of the pheromone appears critical to this orientation as it ceased in zero-vector foragers that were either released off the pheromone or at the pheromone trail's edge. Additionally, manipulation of the celestial compass through the mirroring of the sun's position also affects this orientation, with about half of foragers following the updated sun's position while the other half of foragers maintain orientation to the correct vector direction (likely following the remaining unaltered celestial cues). This change in orientation in the mirrored condition clearly indicates that the celestial compass is in use during zero-vector forager orientation. When zero-vector foragers were tested without the pheromone cue present, they oriented in the opposite compass direction consistent with backtracking behavior, a behavior that is also known to be reliant on the path integrator and until now some unknown aspect of the column (Freas et al. 2019b). Thus, in zero-vector

foragers the presence/absence of the pheromone produced directionally opposite orientation behaviors. Given that these two behavioral outcomes both derive directional information from the celestial compass (Freas et al. 2019b), zero-vector foragers are likely employing a vector memory of their previous foraging trip to orient, while the presence of the pheromone dictates how the forager uses this information.

Cue interaction – inhibiting movement off the column

The pheromone cue also influences foragers' behaviors at the edges of the pheromone trail. Foragers with either a current path integrator (or a vector memory) directing them off the trail, showed no orientation and increased hesitancy at the trail's edge with some foragers choosing to retreat back into the trail, especially in conditions where the remaining vector was near zero. Additionally, we can rule out the effect of small vector distances leading to the lack of vector orientation in these conditions as foragers with the same small 50cm vector that did not correspond with travelling off the pheromone cue remained well oriented. Even foragers with a 3m remaining vector showed some evidence of behavior changes beyond the edge of the trail. In Experiment 1 foragers did not complete their vector distance once they left the pheromone, completing only 57% before the onset of search. Even in the 3m Vector 90° condition, while foragers typically left the pheromone trail after rotation, a minority chose to retreat back and exited in one of the pheromone directions and even some foragers that left initially turned back at the trail's edge. This corresponded with increases in Post-Rotation heading variance observed in this condition compared to 180° rotated foragers. The presence/absence of the pheromone cue along the trail edge and its effect on forager behavior aligns with the observed behavior in the on/off pheromone displacement conditions. Foragers with a larger remaining vector will follow this cue even in the absence of the pheromone, though these foragers will begin to search well before running off the full vector distance. Foragers with only a small remaining vector, reaching an area with no pheromone cue, will engage either in search behavior or backtrack in order to rediscover the trail. In that way the observed retreating at the pheromone edge could be the initial onset of search/backtracking in these foragers. Searching and backtracking behaviors would likely be beneficial for foragers that leave the pheromone after traveling a significant distance of their inbound route as it would increase their chance to rediscover the pheromone trail when most of the trail would be behind them. Conversely foragers with a large amount of their column vector remaining, having momentarily lost the pheromone cue, may benefit more from continued

travel in the vector direction as most of the pheromone trail should still be ahead of them. As such, the presence of the pheromone working as a context cue, directs foragers, both on and off the trail, to engage in behaviors that maximize remaining on the trail and reaching the nest.

Counteracting angular displacement

Animal navigators must contend with environmental forces that often deflect them away from their desired goal, especially those that fly or swim through currents. These navigators have developed a suite of behavioral mechanisms in order to cope with these forces and maintain goalward movement while minimizing displacement on their journey (Chapman et al. 2010, 2011). Ocean bound hatchling loggerhead sea turtles must fight incoming water flows rotating them away from their desired orientation and possess flipper responses to counteract angular displacements by waves (Avens et al. 2003). Over land, flying birds and insects can rely on landmark cues to counter drifting off course due to the wind (Richardson 1991; Srygley and Dudley 2008), but when navigators are naïve or in the open ocean such learned landmark cues are absent and sensing the direction of ocean currents can be difficult (Lohmann et al. 2008; Thorup et al. 2003). Here navigators, such as adult sea turtles drift with currents while maintaining a constant compass heading, leading to non-straight paths across the ocean (Girard et al. 2006; Lushi et al. 2007). Even high-altitude insect migrants, which are more at the mercy of forces like the wind, show evidence of maintaining compass direction during flight under oppositional wind flows (Chapman et al. 2011, 2016).

The challenge of being pushed off course by wind or water currents is not just a problem for swimming and flying animals. Small walking navigators must contend with wind forces that can greatly impact upwind movement and short bursts of wind can fling small animals well off their known route. Ant navigators employ specific behavioral responses to minimize the effect of these forces and maximize their return to known locations (Wystrach and Schwartz 2013). However, it is less understood how small navigators cope with more consistent forces faced by long distance flying or swimming navigators (Chapman et al. 2011).

Orientation to rotating portions of the celestial compass has long been employed in the field of sensory ecology in order to show that the sky's polarization pattern is employed by both walking and flying insects to orient (Mathejczyk and Wernet 2017). These 'polarotactic' behaviors have been demonstrated in many insect species, including tethered walking house flies (*Musca domestica*; von Philipsborn and Labhart 1990) and field crickets (*Gryllus campestris*;

Brunner and Labhart 1987; Henze and Labhart 2007) as well as tethered flying *Drosophila* (Mathejczyk and Wernet 2019) and locusts (*Schistocerca gregaria*; Mappes and Homberg 2004; Homberg 2015) through an increased propensity to turn towards a preferred heading when presented a slowly rotating polarization pattern (1.8 - 7.2°/s). Yet in tethered insects, these turning behaviors were periodic and inconsistent across individuals. These limitations make any assessment of continued celestial compass orientation impossible.

Here, walking ants are able to maintain a heading based on the celestial compass even while being continually angularly displaced at relatively high speeds (~40°/s) off this heading direction. Foragers countered these changes by continuously turning toward the desired compass direction during rotation, similar to the turning behaviors exhibited in tethered insects, yet it is important to note that in the current study even distantly tested ants had access to the full suite of celestial cues, likely improving performance. Even in the Column Head Off-Pheromone condition, where the foragers lagged well behind the rotating vector direction, mean forager body orientation bias was only 34.36°, illustrating impressive performance across a variety of cue presentations and vector states. Even when tested with a small 50cm remaining vector, when vector cue strength would be weak (Wystrach et al. 2015), foragers were adept at maintaining correct body orientation to their celestial compass.

Analysis of performance across the four quarters of the rotation showed no consistent changes across conditions. In all but two conditions, angular error showed no significant differences between quarters, meaning forager orientation to the vector did not deteriorate (or improve) throughout the rotation. In the two conditions where changes in performance were significant, the Clockwise 180° conditions at Nest 1 and Nest 2, these decreases in performance occurred during the middle quarters of the rotation. However, these differences were not observed in most conditions, including the corresponding Counter-Clockwise tests within these conditions, suggesting there was no consistent decrease in performance during the rotation.

Ant navigators are known to be able to deal with quick changes in the cue sets they use to navigate (celestial compass: Freas et al. 2017; Freas et al. 2019a; Reid et al. 2011; landmark cues; Schwarz et al. 2014; though also see: Narendra and Ramirez-Esquivel 2017; Islam et al. 2020). Yet in these studies, the change was immediate and then remained stable. The current study is, to our knowledge, the first instance in which a walking insect has been shown to maintain a consistent compass heading even while the relevant cue remains in flux, similar to the

navigational strategies of long-range swimming and flying migrants (Chapman et al. 2011). The behavioral strategies and coping mechanisms to counteract natural forces such as the wind are ripe for further study in ants.

Conclusions

We found that though the trail pheromone does not contain directionality information, it acts as a context cue that maximizes finding the nest through controlling the expression of behaviors based on the path integrator. When the pheromone was present, foragers oriented to the vector direction regardless of the remaining vector distance, however in the absence of the pheromone cue, foragers' remaining vector dictates when foragers switch behaviors, including searching, backtracking or continued vector orientation. The presence of the pheromone cue triggered vector orientation, both based on the forager's current vector as well as the forager's long-term vector memories. Finally, foragers were highly proficient at adjusting their headings to respond to rotational changes, maintaining a correct compass heading in the vector direction even when this cue was fluid at rotational speeds of $\sim 40^\circ/\text{s}$.

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Conflict of interest

The authors declare no competing or financial interests.

Ethical standards

There are no ethical requirements for working with insects in the United States. Manipulations were non-invasive and all individuals were returned to the nest after testing.

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Tables

Table 1 Circular statistics for forager body orientation during the rotation in Experiment 2a,b

Condition	n	Mean Vector	95% CI		Rayleigh Test		V-test	Vector Compass
			Minus	Plus	Z	p	V	Direction (°)
<u>Experiment 2a On-Column</u>								
Nest 1 180°								
Clockwise	20	17.31°	6°	29°	16.2	<0.001	0.859	<0.001
Counter-Clockwise	20	348.29°	342°	355°	18.7	<0.001	0.948	<0.001
Nest 2 180°								
Clockwise	18	3.43°	353°	14°	15.6	<0.001	0.928	<0.001
Counter-Clockwise	17	329.8°	311°	348°	10.6	<0.001	0.683	<0.001
Nest 1 360°								
Clockwise	20	13.12°	8°	18°	19.3	<0.001	0.957	<0.001
Counter-Clockwise	20	4.72°	358°	12°	19.5	<0.001	0.958	<0.001
<u>Experiment 2a Distant Displacements</u>								
Column Head Off-Pheromone								
Clockwise	16	35.6°	14.3°	56.8°	8.9	<0.001	0.608	<0.001
Counter-Clockwise	15	327.0°	301.9°	352.1°	8.1	<0.001	0.617	<0.001
50% Column On-Pheromone								
Clockwise	16	2.66°	352°	13°	13.9	<0.001	0.931	<0.001
Counter-Clockwise	14	347.2°	336°	358°	12.6	<0.001	0.925	<0.001
<u>Exp. 2b Pheromone-Vector Interaction</u>								
3m Vector 90°								
Clockwise	20	11.0°	354°	28°	12.7	<0.001	0.783	<0.001
Counter-Clockwise	20	351.4°	335°	7°	13.2	<0.001	0.804	<0.001
50cm Vector 90°								
Clockwise	18	2.5°	341°	24°	9.5	<0.001	0.725	<0.001
Counter-Clockwise	19	332.0°	319°	345°	15.0	<0.001	0.784	<0.001
50cm Vector 180°								
Clockwise	16	358.2°	343°	13°	11.9	<0.001	0.863	<0.001
Counter-Clockwise	17	341.7°	328°	355°	12.8	<0.001	0.848	<0.001

Fig. 1. Schematic diagram of two column displacement forager paths and circular histograms in Experiment 1. (A) Diagram of foraging columns and the displacement procedure. Foragers were collected from either the secondary column or the main column and displaced to the main column (grey arrows). Forager paths and circular distributions at the onset of search/grid exit of foragers collected from the (B) main column or (C) secondary column. Black and grey paths denote each forager's travelled route before and after the onset of search respectively. The true nest and main column inbound direction for both circular distributions were set at 0° . Grey arrows in each circular distribution denote the mean vector direction. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

Fig. 2. Diagrams of board rotation procedure in Experiment 2a & 2b (illustrates a 180° clockwise condition). Overhead videos of forager paths were collected on a 60cm × 60cm rotation board. Each forager's path during the (A) Pre-Rotation (light grey), (B) Rotation (black), and (C) Post-Rotation (dark grey) stages were collected by marking the center of the thorax at 0.33sec intervals. During the rotation phase, a second metric was collected, the forager's body orientation in relation to the vector direction, illustrated in panel B. The grey triangle in each panel denotes the nest direction dictated by the inbound pheromone cue while the black triangle signifies the vector direction.

Fig. 3. Forager paths and circular histograms of the 180° and 360° conditions at Nest 1. (A) Sample of individual forager paths during the Pre-Rotation (light grey), Rotation (black), and Post-Rotation (dark grey) stages (for all paths, see Fig. S1 and Fig. S4). Grey triangles denote the change in the vector compass direction Pre-Rotation and Post-Rotation, while the black semi-circle/circle illustrates the movement of the vector direction (rotation of vector direction is opposite the direction of the board's rotation). (B) Circular histograms for mean body orientation during the rotation (vector direction set at 0°). Black arrows in each panel denote the mean vector direction. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

Fig. 4. Forager paths and circular histograms of the distant 180° conditions. 50% Column Foragers were collected from the mid-point of the column with half their remaining vector. Column Head Foragers were collected at the end of the column as foragers re-entered the column from the foraging fan and had their full column vector remaining. (A) Sample of individual forager paths during the Pre-Rotation (light grey), Rotation (black), and Post-Rotation (dark grey) stages (for all paths, see Fig. S5 and Fig. S7). The grey triangles denote the change in the vector compass direction Pre-Rotation and Post-Rotation, while the black semi-circle illustrates the movement of the vector direction (opposite board's rotation). (B) Circular histograms for mean body orientation during the rotation (vector direction set at 0°). Black arrows in each circular distribution denote the mean vector direction. *n*, number of individuals; $\bar{\theta}$, mean vector; *r*, length of the mean vector.

Fig. 5. Forager paths and circular histograms of the 90° conditions. (A) Sample of individual forager paths during the Pre-Rotation (light grey), Rotation (black), and Post-Rotation (dark grey) stages (for all paths, see Fig. S8 and Fig. S9). The pheromone trail was restricted to the center 30cm of the board (grey area). Grey triangles denote the change in the vector compass direction Pre-Rotation and Post-Rotation, while the black quarter-circle illustrates the movement of the vector direction (opposite board's rotation). (B) Circular histograms for mean body orientation during the rotation (vector direction set at 0°). Black arrows in each circular distribution denote the mean vector direction. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

Fig. 6. Forager paths in the Zero-Vector conditions in Experiment 3. Foragers were collected just before entering the nest, held for 5min and displaced distantly to one of two 120cm × 60cm testing boards; an ‘on-pheromone’ board where the pheromone had been deposited in the center 30 cm (grey area) and an ‘off-pheromone’ control board. (A) Zero-Vector On-Pheromone, (B) Zero-Vector 180° Rotated Pheromone, (C) Zero-Vector Off-Pheromone. In each panel the black circle marks the release position at the centre of the board, the black triangle denotes inbound column-to-nest compass direction (recent inbound vector) while the grey triangle indicates the inbound direction dictated by the pheromone cues on the board (except panel C where no pheromone was present). *n*, number of individuals.