**Pheromone cue triggers switch between vectors in the desert harvest ant, *Veromessor*
*pergandei***

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Abstract

The desert harvester ant (*Veromessor pergandei*) employs a mixture of social and individual navigational strategies at separate stages of their foraging trip. Individuals leave the nest along a pheromone-based column, travelling 3-40m before spreading out to forage individually in a fan. Foragers use path integration while in this fan, accumulating a direction and distance estimate (vector) to return to the end of the column (column head), yet foragers' potential use of path integration in the pheromone-based column is less understood. Here we show foragers rely on path integration both in the foraging fan as well as while in the column to return to the nest, using separate vectors depending on their current foraging stage in the fan or column. Returning foragers displaced while in the fan oriented and travelled to the column head location while those displaced after reaching the column travel in the nest direction, signifying the maintenance of a two-vector system with separate fan and column vectors directing a forager to two separate spatial locations. Interestingly, the trail pheromone and not the surrounding terrestrial cues mediate use of these distinct vectors, as fan foragers briefly exposed to the pheromone cues of the column in isolation altered their paths to a combination of the fan and column vectors. The pheromone cue acts as a contextual cue triggering both the retrieval of the column vector memory and its integration with the forager's current fan vector.

Keywords: navigation; celestial compass; path integration; local vectors; pheromone trails

Introduction

Animals employ a variety of navigational strategies to reach goal locations. Chief among these strategies, evidenced across many animal groups, is path integration (Mittelstaedt and Mittelstaedt 1982; Wehner et al. 1996; Collett and Collett 2000; Wehner and Srinivasan 2003; Etienne and Jeffery 2004). An animal navigator's path integration system provides it a continuously updating spatial estimate of its starting location, typically its home or nest, allowing for an efficient return trip from any point along the foraging trip (Collett and Collett 2000; Wehner and Srinivasan 2003).

In foraging ants, the path integration system involves the coupling of a distance estimate, tracked primarily through a step-counter or pedometer (Wittlinger et al. 2006), with directional estimates informed by the ant's celestial compass, which consists of multiple cue sets including the sun's position and the overhead polarisation pattern of the sky (Wehner and Müller 2006; Wehner 2008). The resulting nest-ward vector guides the forager's inbound travel back to the nest area without having to retrace the outbound route. As the path integration system is prone to the accumulation of error, foragers often must search for the nest entrance through a backup strategy termed systematic search (Wehner and Srinivasan 1981; Müller and Wehner 1994, Schultheiss et al. 2015). In solitary foraging ants the path integrator pointing to the nest, termed the global vector, is known to be active throughout the foraging trip, resetting at the end of each foraging bout only after the forager enters the nest (Knaden and Wehner 2006).

When species inhabit cluttered environments that contain many terrestrial landmarks, foragers also acquire and retain terrestrial cue information by collecting goal-oriented views of the surrounding landmark panorama, both while at the nest site and along foraging routes to navigate between locations (Narendra 2007; Collett et al. 2013; Cheng et al. 2009; Graham and Cheng 2009; Legge et al. 2014; Freas and Spetch 2019; Freas et al. 2019a). As foragers learn the visual cues along their foraging route, they develop stereotypical routes around obstacles while returning to the nest, often steering away from the nest direction and the global vector during these detours (Collett et al. 1992; Bisch-Knaden and Wehner 2001; Wystrach et al. 2011; Mangan and Webb 2012). During these periods of route following in well-known locations, ant and bee foragers are thought to suppress the use of their global vector with a number of 'local vectors' along the habitual foraging path, encoding both directional and distance estimates of separate segments of the route (Srinivasan et al. 1997; Menzel et al. 1998; Collett et al. 1998;

Collett and Collett 2009; Legge et al. 2010; Collett and Collett 2015). Foragers learn these local vectors in association with panorama views along the foraging route and these route segment memories can be subsequently triggered by the presentation of this familiar panorama (Collett et al. 1998; Collett et al. 2002; Knaden et al. 2006). Importantly, local vectors are independent memories of the route segment triggered by familiar views, not a reloading of the global vector and can be retrieved regardless of the current path integrator state (Collett et al. 1998; Bisch-Knaden and Wehner 2001; Collett et al. 2002; Collett and Collett 2015). Some examples of local vectors have pointed to the use of celestial compass and odometry memories to travel these route segments (Collett and Collett 2015), though the critical presence of the familiar panorama means that the term ‘local vector’ may also include foragers aligning to view-based memories at known panoramas without retaining memories of associated segment distances (odometer) or directions through the celestial compass (Baddeley et al. 2012; Webb 2019; though also see: Knaden et al. 2006; Collett and Collett 2015; Fernandes et al. 2015).

Recently, the use of multiple vector memories directed by celestial and odometer cues has been explored in the desert harvester ant (*Veromessor pergandei*), as foragers of this species show evidence of path integration originating from a non-nest site (Plowes et al. 2019). *V. pergandei* forages with a column and fan structure, using both social and individual navigational cues in separate stages of the foraging trip. Foragers leave the nest in a pheromone-marked column. After several meters (ranging from 3 to 40 meters), this column ends and past this location individuals spread out to forage individually in a foraging fan (Plowes 2012; Plowes et al. 2013, 2014). Once a piece of food is collected, fan foragers use path integration to return to the column head and as foragers re-enter the pheromone column they change their heading direction toward the nest entrance, following the column to the nest (Plowes et al. 2019). Previous work has shown that returning fan foragers rely on path integration-based cues that return the forager to the column head and not the nest entrance (Plowes et al. 2019; Freas et al. 2019b). This behaviour has been theorised to indicate that foragers only begin to accumulate a path integrator after leaving the pheromone trail. However, there are multiple recent lines of evidence that suggest foragers instead path integrate both while in the fan and the column. First, fan forager paths to the column head are biased in the nest direction, suggesting that foragers maintain a nest-ward vector in the column, which is suppressed during fan navigation (Plowes et al. 2019). Furthermore, the presence of a column vector is supported by recent findings showing

that foragers both attend to celestial cues and accumulate distance estimates while on the pheromone-based column. Foragers collected from the column head orient in the nest compass direction when displaced distantly (Plowes et al. 2019), however this directed behaviour ceases when the sun's position is mirrored and headings become random (Freas et al. 2019c). *V. pergandei* foragers also actively attend to both the sun's position and the overhead polarisation pattern while returning in the column (Freas et al. 2019b). Finally, backtracking, a behaviour largely dependent on foragers running off their remaining vector (Wystrach et al. 2013), is exhibited in this species only when foragers have completed over 75% of the column distance, suggesting the maintenance of a distance estimate in the column (Freas et al. 2019c; Plowes et al. 2019). Given these findings, fan and column foragers likely accumulate two distinct vectors during their foraging trip, a column vector and a fan vector. However, it remains unknown what mechanism underlies the switch between these vectors and if this foraging ecology aligns with the previously discussed route local vector use exhibited in solitarily foraging species. Critical for local vector use in these species is the familiar panorama along well-known routes, but *V. pergandei* has shown no evidence of orienting to or using terrestrial cues to return to the nest, despite actively using visual compass cues and inhabiting a cluttered environment with many available visual landmarks (Freas et al. 2019b, c).

To characterise the use of separate vectors in the fan and column, we tested which mechanism causes fan foragers to switch from navigation to the column head to navigation to the nest entrance. We collected fan foragers with the largest observed directional difference between their fan and column vectors ($\sim 90^\circ$) and recorded their initial headings and return paths with and without exposure to the column. As fan collected foragers exposed to the column shifted their headings and paths to the global vector (the compass direction from the collection site to the nest), we also tested if this heading change was controlled by the panorama cues of the column or the pheromone trail. Forager heading and path shifts were shown to be elicited by the cues of the pheromone based foraging column even in isolation to all other cue sets, revealing that the pheromone cue triggers the switch between fan and column vectors and support *V. pergandei*'s column and fan foraging structure.

General Methods

Study sites and species

Testing was conducted during October to November 2018 and April 2019 on four *Veromessor pergandei* nests. Nests 1 and 2 were located at the South Mountain Municipal Park in Phoenix Arizona, USA (33°19'43.10" N, 112°01'02.60" W) and these nests were separated by ~150m. Nest 3 and Nest 4 (~375m apart) were located ~50km to the west, within the Verrado Temporary Trail System in Buckeye, Arizona, USA (33°29'50.13" N, 112°31'57.36" W). *V. pergandei* nests are common throughout the Mojave and Sonoran deserts and their habitats are visually complex, consisting of scattered bushes, trees and cacti (Supplemental Figure 1). During testing, only returning foragers which had a visible food piece were tested in order to ensure homeward motivation. After testing, foragers were collected to prevent retesting and returned to the nest entrance after testing was complete.

Statistical Analysis

In all experiments, forager initial headings and paths were analysed with circular statistics (Batschelet 1981; Zar 1998) using Oriana Version 4 (Kovach Computing Services TM). Initial heading directions were analysed using Rayleigh Tests to determine if data was non-uniform. As forager headings in all conditions across all seven experiments were non-uniform, signifying orientation in a direction, these results were collected in Table 1 and Table 2. Data was analysed using V-tests to determine if headings were grouped in a predicted direction (fan vector, column vector, global vector, or true nest direction). As V-tests are statistically weak, we verified forager orientation direction by testing whether the predicted directions fell within the 95% confidence interval of observed headings. In Experiments 2 through 6, comparisons of forager headings and paths between conditions were calculated using Watson–Williams F-tests with Holm–Bonferroni corrections for multiple comparisons.

For Experiments 2-6, when analysing fan collected forager data, headings were tested for orientation to four separate predicted directions: true nest, fan vector, column vector, and global vector directions. The true nest position was designated as the nest's directional position from the test site. The fan vector direction was selected as $\pm 10^\circ$ from the fan to column head compass direction (100° or 260°) as fan foragers were collected from a 20° range. The column vector direction was designated as the column-head-to-nest compass direction. The global vector direction in each condition was defined as the combination of the remaining fan vector and the column vector (the compass direction to the nest from the collection site) in each condition.

For Experiment 4, forager paths were digitized using GraphClick (Arizona Software) and analysed by calculating the foragers' heading directions and path lengths before the onset of systematic search. The end of navigation through path integration and the onset of search was defined as the first sharp $\geq 60^\circ$ turn followed by the forager continuing in the new direction for at least 25cm. This procedure was implemented to exclude turns during detours around small obstructions at the testing site. Additionally, turns that occurred at the beginning of the released ant's path (within 50cm) were excluded to allow the forager to choose a heading. Forager headings were calculated and compared between conditions both as the mean heading direction before search and the forager's position at the onset of search using Watson–Williams F-tests. Forager path lengths at the onset of search were analysed between conditions using Kruskal–Wallis rank sum tests with post hoc Dunn comparisons adjusted using the Holm–Bonferroni method for multiple comparisons.

Experiment 1 - Local displacements around column head

a. Methods

As recent work has already concluded that foragers do not orient to the nest entrance using terrestrial cues (Freas et al. 2019c), our first tests were conducted to determine whether foragers use terrestrial cues to orient to the column head. Local displacement testing was conducted at Nest 1 and Nest 4 around the column head (column length, 7.2m Nest 1 and 15.5m at Nest 4. Four displacement sites were designated 3.5m from the column head: two were located $\pm 90^\circ$ off the foraging column (90° and 270°), one was located 180° at 3.5 m past the column head (in line with the column), and the fourth was a 0° displacement site, located on a board raised 15cm above the foraging column (Figure 1a). A fifth displacement site was designated 1m past the nest entrance (Figure 1a). Inbound foragers were collected at the column head and displaced to one of these local displacement sites. At each site, foragers were released on a wooden board with a goniometer painted on its surface, an instrument used to measure the angle of a forager's initial orientation. This goniometer consisted of a 60-cm-diameter circle separated into 24, 15° wedges. Initial heading directions of foragers were measured by crossings at 30cm. At the 90° , 270° , 180° and 1m Past sites a goniometer was placed and levelled on the ground, while at the 0° displacement site the goniometer was raised 15cm above the foraging column using four flat top edging stakes (Figure 1a).

In a second set of local displacements, foragers at Nest 1 and Nest 4 were collected from the foraging column just before they entered the nest, termed ‘zero vector’ foragers as their path integrator would be near zero and uninformative for orientation (Figure 2a). These individuals were transferred to either the 90° or 270° sites (Figure 2a) where they were released on the goniometer and their initial headings were recorded.

b. Results

Foragers collected either from the column head or the nest showed no evidence of orientation to the column head except when this direction also aligned with the forager’s column vector (180° site). In all of the local displacement conditions (Figure 1a) at both Nest 1 and Nest 4, foragers collected from the column head oriented in the column vector direction (column-head-to-nest compass direction). Forager headings were oriented in the column vector direction regardless of release site (V-test and 95% CI; Table 1; Figure 1b-k). In contrast, zero vector foragers collected just before entering the nest and released locally (Figure 2a) exhibited headings that were not oriented to either the column head or column vector directions (Table 1; Figure 2b-e). These foragers instead oriented opposite the column vector (V-test at 0°; Nest 1 90° site; $V = 0.58$; $p < 0.001$; Nest 1 270° site; $V = 0.64$; $p < 0.001$; Nest 4 90° site; $V = 0.87$; $p < 0.001$; Nest 4 270° site; $V = 0.83$; $p < 0.001$) consistent with backtracking behavior. Further analysis of these initial headings showed that the backtracking direction (0°) was within the 95% CI of headings at Nest 4, but at Nest 1 the backtracking direction fell just outside the 95% CI of headings in both the 90° and 270° tests (with 95% CI ending at 357° and 2° respectively; Figure 2b,c).

Column head foragers in all conditions appeared to rely heavily on their path integrator, orienting in the direction expected if following a vector to the nest entrance and showed no evidence of being directed to the column head. Zero vector foragers showed no evidence of using terrestrial cues or other local cues to return to the column head or nest, instead orienting consistent with backtracking behaviour (Wystrach et al. 2015; Freas et al. 2019c).

Experiment 2 - Cross Fan Displacements

a. Methods

These tests were conducted to confirm that foragers in the fan rely on a fan vector to return to the column head even when displaced locally, and to test whether exposure to the column would alter this behaviour. At Nest 1, two collection areas were designated in the foraging fan 2-3m

from the column head (column length, 6.5m) and between 70-90° and 270-290° off the nest-to-column-head compass direction (Figure 3ab; dark grey area). Inbound fan foragers were collected from these areas and designated to one of three condition groups. In the first control condition, the Cross Fan Control, foragers were displaced to the opposite side of the foraging fan onto a goniometer 3m from the column head and $\pm 90^\circ$ off the nest-to-column-head compass direction (Figure 3a) where individual foragers' headings were measured. Foragers in the second control condition, the Fan Exposure, were collected identically to the previous condition but were released onto the foraging fan for 20sec and then re-collected and tested on the opposite side of the foraging fan. In the final condition, Column Exposure, foragers were collected identically to previous conditions but after collection were released onto the mid-point of the column, equidistant from the column head and the nest entrance (Figure 3b), for 20sec and then tested identically to previous conditions.

b. Results

At Nest 1, Cross Fan Control foragers (270° to 90° and 90° to 270°; Figure 3a) exhibited headings oriented to the fan vector direction, at 260° and 100° respectively (V-test and 95% CI; Table 2; Figure 3c,d) and not the column vector or the true nest direction (Table 2). In the 270° to 90° Cross Fan Control, the V-test did not allow to reject the global vector direction at 202°, but this direction fell well outside the 95% CI of headings which ended at 248.5° (Table 2).

Fan collected foragers released on the column for 20sec and then displaced across the fan (270° to 90° and 90° to 270°; Figure 3a) oriented toward the global vector direction (V-test and 95% CI; Table 2; Figure 3e,f) and were significantly different from the Cross Fan Control headings in both the 270° to 90° (Watson-Williams F test; $F_{(1,58)} = 23.8$; $p < 0.001$) and 90° to 270° conditions (Watson-Williams F test; $F_{(1,58)} = 66.8$; $p < 0.001$). V-tests did not differentiate among the four predicted directions (Table 2) yet in both conditions only the global vector directions (202° and 158° respectively) fell within the 95% CI of headings.

Fan Exposure foragers displaced across the fan did not exhibit the same heading changes as column exposed individuals, with mean headings that were not significantly different from Cross Fan Controls (Watson-Williams F test; 90° to 270°; $F_{(1,58)} = 2.5$; $p = 0.12$; 90° to 270°; $F_{(1,53)} = 2.5$; $p = 0.12$) but were significantly different from Column Exposure foragers (Watson-Williams F test; 90° to 270°; $F_{(1,58)} = 26.8$; $p < 0.001$; 90° to 270°; $F_{(1,53)} = 34.3$; $p < 0.001$). For fan exposed foragers, the V-test did not show forager headings grouped in the true nest or column

head directions but could not differentiate between the three vector directions; Fan, Column, or Global (Table 2; Figure 3g,f). However, only the fan vector (100°) direction was within the 95% CI of headings in the 90° to 270° Fan Exposure condition (Table 2; Figure 3h). In the 270° to 90° Fan Exposure condition, these foragers showed a slight bias in the nest compass direction and the fan vector (260°) direction was just outside the 95% CI of headings, ending at 257.5° (Figure 3g). The observed small bias to the nest compass direction in fan collected ants is consistent with previous work (Plowes et al. 2019). Overall, foragers collected from the fan, similar to column head foragers, relied heavily on their path integrator, however this path integrator pointed to the column head (before displacement) rather than the nest entrance. Exposure to the pheromone-based column caused a change in this orientation behaviour with foragers shifting their headings in the direction of the global vector (column vector plus fan vector).

Experiment 3 - Distant Displacements

a. Methods

Distant Displacement tests were conducted at Nests 2 and 3 to explore if the same orientations and heading shifts after column exposure occurred even when the foragers were displaced far from the nest. At Nest 2, fan foragers were collected (fan collection area 2-3m from column head and 270-290° off the nest-to-column head compass direction; column length, 9.8m) and designated as either Fan Control or Column Exposure foragers. A third forager group, Column Head foragers, were collected from the end of the column. Fan Control and Column Head foragers were immediately displaced to a distant release site (92m from the nest) while Column Exposure foragers were released on the column for 20sec, then re-collected and released at the distant site onto a wooden board with a 60cm diameter reference circle. Forager crossings, rounded to the nearest degree, were recorded using a high-definition camera at 30 fps 3840 × 2160 pixels image size (GoPro 5TM) positioned 60 cm above the reference circle.

A mirror replicate (70-90° fan collection) of these conditions was conducted at Nest 3 with a 10m column length and a distant displacement site 50m from the nest entrance. The fan at this nest was smaller and fan foragers were collected from 1-1.5m from the column head.

b. Results

These heading patterns persisted when foragers from Nests 2 and 3 were displaced distantly instead of locally across the fan. At Nest 2, Fan Control forager headings were oriented only in

the fan vector direction (V-test and 95% CI; Table 2; Figure 4a), while Column Head foragers were oriented to the column vector direction and not the fan vector direction (V-test and 95% CI; Table 2; Figure 4b). Column Head foragers also exhibited significantly different mean headings compared to the Fan Control condition (Watson-Williams F test; $F_{(1,43)} = 31.0$; $p < 0.001$). Column exposed (20sec) forager headings were in an intermediate direction between the fan vector and column vector direction (Table 2; Figure 4c) and while V-tests indicated grouping in all three tested directions, all these directions were outside the 95% CI of headings (132.6° - 159.6°) with the global vector being just outside at 165° indicating a bias in the fan vector direction. Additionally, the mean heading of Column Exposure fan foragers was significantly different from both the Fan Control (Watson-Williams F test; $F_{(1,39)} = 17.0$; $p < 0.001$) and Column Head conditions (Watson-Williams F test; $F_{(1,42)} = 5.7$; $p = 0.021$).

At Nest 3 where foragers were collected from 270° on the fan, Fan Control foragers were oriented only in the fan vector direction (V-test and 95% CI; Table 2; Figure 4d). Foragers displaced from the column head were oriented in the column vector direction (V-test and 95% CI; Table 2; Figure 4e). Given the small fan size at this nest there was only a 9° difference between the column vector and global vector compass directions making statistically distinguishing headings between these directions difficult. These column head collected foragers were also statistically oriented in the global vector direction (V-test and 95% CI; Table 2). Mean vectors between the Fan Foragers and Column Head foragers were significantly different (Watson-Williams F test; $F_{(1,58)} = 48.4$; $p < 0.001$). Column Exposure foragers were grouped in all three predicted directions but all three directions were outside the 95% CI (Table 2) with column exposed foragers once again showing a slight bias in the fan vector direction vs the expected global vector direction. The mean vector of Column Exposure foragers was significantly different from the control (Watson-Williams F test; $F_{(1,53)} = 62.6$; $p < 0.001$) but not from Column Head foragers (Watson-Williams F test; $F_{(1,53)} = 2.2$; $p = 0.14$).

Identically to cross fan displacements, distantly displaced foragers collected in the fan oriented in the fan vector direction, while column exposed forager headings were closest to the global vector direction. These headings at a distant site suggest foragers rely on path integration and not local cues around the column head when orienting.

Experiment 4 - Forager Paths

341 *a. Methods*

342 To determine if the observed differences in initial orientations persisted after 30cm we collected
343 foragers' paths at both Nest 1 and Nest 3. At Nest 1, a 4m × 5m grid of 1m squares was erected
344 in a clearing 50m from the nest entrance using pegs and string. After this grid was erected the
345 column of Nest 1 shifted 11° clockwise and then remained stable during testing. Due to this
346 change, the column to nest compass direction in this test was 191°. Fan Control and Column
347 Exposure foragers were collected from an area 2-3m from the column head and at 270-290° off
348 the nest-to-column-head compass direction, while Column Head foragers were collected from
349 the column head (column length = 4.8m). Fan Control and Column Head foragers were displaced
350 to the distant testing grid while Column Exposure foragers were first released onto the foraging
351 column for 20sec, re-collected and then displaced. At the testing grid, foragers were released and
352 their paths were recorded using graph paper. Each forager was recorded until it left the grid or
353 after 5min.

354 As a number of foragers in each condition left the grid before the onset of search, a
355 replicate (70-90° fan collection) of these three path conditions was conducted at Nest 3 with a
356 10m column length, a 1-1.5m fan collection area and a distant displacement site 62m from the
357 nest entrance. At this distant site an 8m × 3m grid of 1m squares was erected and Fan Control,
358 Column Exposure and Column Head foragers were collected and tested identically to Nest 1.

359 *b. Results – Path direction*

360 At Nest 1, Fan Control foragers' mean path directions were directed only in the fan vector
361 direction at 111° (V-test and 95% CI; Table 2; Figure 5a,b; light grey). When analysing the onset
362 of search direction of these foragers, headings were grouped in the fan vector direction but were
363 biased in the nest compass direction with the predicted fan vector direction falling outside the
364 95% CI of headings which ended at 124° (Table 2; Figure 5c). Both Column Head foragers'
365 mean path directions and the onset of search directions were directed only in the column vector
366 direction at 191° (V-test and 95% CI; Table 2; Figure 5a-c; dark grey). Column Head foragers'
367 directional headings were significantly different from the Fan Forager control when comparing
368 both the mean vectors of path direction (Watson-Williams F test; $F_{(1,27)} = 258.2$; $p < 0.001$;
369 Figure 5b) and onset of search direction (Watson-Williams F test; $F_{(1,27)} = 262.2$; $p < 0.001$;
370 Figure 5c). Column Exposed foragers exhibited both mean path directions and onset of search
371 directions that were only oriented in the global vector direction at 162° (V-test and 95% CI;

Table 2; Figure 5a-c, black). Additionally, Column Exposed fan foragers' mean vector directions were significantly different from both the Fan Forager control and Column Head foragers when comparing mean path direction (Watson-Williams F test; Fan Foragers; $F_{(1,27)} = 53.0$; $p < 0.001$; Column Head; $F_{(1,28)} = 57.1$; $p < 0.001$; Figure 5b) and the onset of search direction (Watson-Williams F test; Fan Foragers; $F_{(1,27)} = 91.5$; $p < 0.001$; Column Head; $F_{(1,28)} = 52.2$; $p < 0.001$; Figure 5c).

At Nest 3, both Fan Control forager mean path and search onset directions were grouped in the fan vector direction at 260° (Table 2; Figure 6a-c, light grey), but both were biased in the nest compass direction with the predicted direction falling outside the 95% CI of headings which ended at 245° for both measurements (Figure 6b,c). Column Head foragers' directional headings were oriented in the column vector direction at 180° (V-test and 95% CI; Table 2; Figure 6a-c, dark grey) and the mean vectors of both measurements were significantly different from the Fan Forager control (Watson-Williams F test; mean path direction; $F_{(1,28)} = 336.2$; $p < 0.001$; Figure 6b; onset of search direction; $F_{(1,28)} = 173.2$; $p < 0.001$; Figure 6c). Column Exposed foragers exhibited both mean path directions and onset of search directions that were grouped in the global vector direction at 189° (Table 2; Figure 6a-c, black) but forager headings were biased away from the column to nest compass direction with the predicted global vector direction outside the 95% CI of both the mean path (ends at 202° ; Figure 6b) and onset of search direction (ends at 196° ; Figure 6c). The mean vectors of both measurements were significantly different from both the Fan Control (Watson-Williams F test; mean path direction; $F_{(1,28)} = 82.7$; $p < 0.001$; onset of search direction; $F_{(1,28)} = 76.6$; $p < 0.001$) and Column Head foragers (Watson-Williams F test; mean path direction; $F_{(1,28)} = 58.4$; $p < 0.001$; Figure 6b; onset of search direction; $F_{(1,28)} = 48.5$; $p < 0.001$; Figure 6c).

c. Results – Path length

At both Nest 1 and Nest 3, Path length before the onset of search also significantly differed between conditions (Kruskal-Wallis test; Nest 1; $KW = 12.45$; $p = 0.002$; Nest 3; $KW = 22.56$; $p < 0.001$). At Nest 1, Dunn post hoc tests with Holm corrections showed no significant difference ($p = 0.233$) between path lengths in Column Head foragers (mean $\pm$ S.E.; $\mu = 3.03 \pm 0.14$ m) and Column Exposure fan foragers (mean $\pm$ S.E.; $\mu = 3.33 \pm 0.11$ m). Fan Control foragers' mean path lengths (mean $\pm$ S.E.; $\mu = 2.59 \pm 0.15$ m) were significantly different from both Column Head foragers (Dunn's test; $p = 0.049$) and Column Exposure fan foragers (Dunn's test; $p =$

0.001). However, 21% of Fan Control, 26.7% of Column Head and 40% of Column Exposure foragers left the testing grid before the onset of search, limiting our ability to assess path length at this nest.

When we expanded the testing area during testing at Nest 3, Column Head (mean $\pm$ S.E.; $\mu = 2.31 \pm 0.21\text{m}$) and Column Exposure foragers (mean $\pm$ S.E.; $\mu = 3.23 \pm 0.20\text{m}$) did significantly differ in path length (Dunn's test; $p = 0.028$). Additionally, Fan Control foragers' mean path lengths (mean $\pm$ S.E.; $\mu = 1.40 \pm 0.10\text{m}$) were significantly shorter than both Column Head foragers (Dunn's test; $p = 0.027$) and Column Exposure fan foragers (Dunn's test; $p < 0.001$). Here, 6.7% of Fan Control, 0% of Column Head and 13.3% of Column Exposure foragers left the testing grid before the onset of search.

Both forager path directions and path lengths suggest fan foragers use a fan vector to return to the column head site (with a bias in the nest compass direction) and then begin searching, while column head foragers orient and travel in the compass direction of the nest entrance. Interestingly, foragers collected in the fan and exposed to the column navigate differently to both other groups, travelling in the global vector direction and exhibiting the longest path lengths before the onset of search, suggesting the integration of both the fan and column vector after column exposure.

Experiment 5 - Column panorama exposure

a. Methods

To test if the panorama cues at the column mediated the observed shifts in column exposed foragers, we tested foragers orientations when exposed to the column panorama during testing. At Nest 1, fan foragers were again collected from 2-3m from the column head and $\pm 70-90^\circ$ off the nest to column head compass direction (column length = 6.5m). Fan Control foragers were released in the foraging fan for 20sec, re-collected and then tested above the foraging column on a raised goniometer (15cm off the ground) where initial headings were recorded (Figure 7a). This goniometer was placed at the mid-point of the column, 3.5m from both the column head and the nest entrance and raised 15cm above the foraging column using four flat top stakes identical to Experiment 1 (Figure 7a,b). Column Exposure foragers were collected identically to Fan Control foragers but were released onto the foraging column (below the board) for 20sec before testing above the column (Figure 7b). Finally, Column Head foragers were collected at the

column head, released for 20sec on the column and then tested above the column identically to previous conditions (Figure 7b).

b. Results

Fan foragers collected in the fan at either the 270° or 90° collection area, released in the fan for 20sec and then re-collected and released 15cm above the column (Figure 7a) exhibited headings oriented in their fan vector directions (V-test and 95% CI; Table 2; Figure 7c,d) and not the column vector, true nest or global vector directions (Table 2). Fan foragers collected from the same locations in the fan (270° or 90°), released on the column for 20sec, then re-collected and tested 15cm above the column (Figure 7b) exhibited headings oriented in the global vector direction and not the fan vector direction (V-test and 95% CI; Table 2; Figure 7e,g). In the 270° Column Exposure condition, forager headings were also not oriented to the column vector direction (95% CI), however in the 90° Column Exposure condition, forager headings were also considered oriented to the column vector direction at 180° (95% CI; Table 2). Column Head and Exposure foragers also exhibited headings that were directed to the column vector direction and not the fan vector or global vector directions (V-test and 95% CI; Table 2; Figure 7f). Additionally, both 270° Column Exposure and 90° Column Exposure foragers exhibited mean headings that significantly differed from both their respective fan controls (270° Column Exposure; Watson-Williams F test; $F_{(1,57)} = 31.90$; $p < 0.001$; 90° Column Exposure; Watson-Williams F test; $F_{(1,58)} = 43.17$; $p < 0.001$) and Column Head foragers (270° Column Exposure; Watson-Williams F test; $F_{(1,88)} = 13.35$; $p < 0.001$; 90° Column Exposure; Watson-Williams F test; $F_{(1,88)} = 3.99$; $p < 0.049$). These results indicate that the panorama cues of the column do not mediate the observed shifts in vector use, as foragers collected in the fan and displaced just above the column still oriented in the fan vector direction, while foragers exposed directly to the column and tested identically do shift their heading direction.

Experiment 6 - Pheromone tests

a. Methods

To determine if the column's pheromone cues mediated the observed forager heading shifts, we tested foragers with limited access to other cues while on the column by placing them under an aluminum phial. We additionally tested if the pheromone trail presented alone would lead to the observed shifts by exposing foragers to the soil of the column within a phial. At Nest 1, fan

foragers were collected in an identical collection area (+70-90° off the column-head-to-nest compass direction and 2-3m from the column head; column length, 5.2m) and separated into four groups: Fan Control, Column Covered, Empty Phial and Pheromone Phial conditions. In the Fan Control condition, foragers were collected from the fan and then released onto a goniometer at a distant test site ~70m from the nest entrance. In the Column Covered condition, collected fan foragers were released on the midway point of the column and covered with an aluminium 6cm diameter phial for 20sec. This phial blocked individuals from exposure to other foragers, from visual (terrestrial and celestial) cues and the ability to move along the column in any direction. After 20sec, these foragers were immediately re-collected, and tested identically to the Fan Control condition. In the Empty Phial condition, fan foragers were individually placed into an empty 6cm diameter phial for 20sec and then released and tested distantly identically to the previous conditions. Finally, in the Pheromone Phial condition, soil was collected from the column area at the midpoint of the column length and spread along the bottom of a phial to create a ~2cm base of column soil. Individual fan foragers were collected and held in the phial for 20 sec then tested at the distant test site identically to the Empty Phial condition.

b. Results

At the distant testing site, Fan Control headings were oriented in the fan vector direction and not the column vector or global vector directions (V-test and 95% CI; Table 2; Figure 8a). Column Covered fan foragers were also oriented but only the global vector direction (153°) was within the 95% CI of headings (Table 2; Figure 8b). Additionally, the mean vector of headings was significantly shifted toward the global vector direction compared to the Fan Control (Watson-Williams F test; $F_{(1,59)} = 85.9$; $p < 0.001$). Empty Phial exposed foragers were oriented in the direction of the fan vector and not the column vector or global vector directions (V-test and 95% CI; Table 2; Figure 8c) and the mean vector of headings was not significantly different from the Fan Forager control (Watson-Williams F test; $F_{(1,58)} = 0.19$; $p = 0.667$). Pheromone Phial exposed foragers were oriented only in the global vector direction (V-test and 95% CI; Table 2; Figure 8d). Additionally, the mean vector of headings was significantly shifted toward the global vector direction compared to the Fan Forager control (Watson-Williams F test; $F_{(1,59)} = 85.4$; $p < 0.001$) and was not significantly different from Column Covered foragers (Watson-Williams F test; $F_{(1,58)} = 3.12$; $p = 0.083$). The Column Covered condition suggests that during the 20sec column exposure period, foragers do not need to: interact with other foragers, travel along the

column before testing, or see the overhead celestial cues during exposure, suggesting these cues are not critical to the observed heading shifts and the likely use of vector memory mediated by the pheromone cues of the column. In support of this theory, when the soil of the column was presented alone, this was sufficient to produce the observed heading shifts, suggesting that the pheromone cue elicits the integration of separate vector memories while foraging.

Experiment 7 - Goniometer Control

a. Methods

To confirm that column forager headings were not due to a potential accumulation of chemical cues on the board in the goniometer conditions, we conducted a control experiment at Nest 1. A goniometer was placed on the ground 90° off column and 3.5m from the nest site and Column Head foragers were collected and tested (column length = 6.5m). After 40 foragers were tested, the goniometer board was rotated 180° in place and a further 30 foragers were tested on this rotated board.

b. Results

Headings of the first 40 foragers, in the Initial Goniometer condition (before 180° goniometer rotation) were non-uniform (Rayleigh Test; $Z = 34.0$; $p < 0.001$) and grouped around the column vector direction (V-Test; $V = 0.92$; $p < 0.001$; Supplemental Figure 2a). Additionally, the column vector direction was within the 95% CI of headings (Supplemental Figure 2a). After the 180° goniometer rotation, in the Flipped Board condition, initial headings were still non-uniform (Rayleigh Test; $Z = 20.7$; $p < 0.001$) and headings were still grouped in the column vector direction, now at 180° on the goniometer (V-Test; $V = 0.82$; $p < 0.001$; Supplemental Figure 2b) and the column vector direction remained within the 95% CI of headings. Results suggest that the potential accumulation of pheromone cues on the testing board were unlikely to produce the observed results in Experiments 1-6.

Discussion

Summary

Previous work on *V. pergandei* has highlighted that forager's path integration system is active both while navigating alone in the fan and while on the pheromone column (Plowes et al. 2019; Freas et al. 2019b,c). Here we show that *V. pergandei* foragers navigate in a two-vector system

accumulating separate path integration-based vectors while in the pheromone-based column and in the foraging fan. Interestingly, we show for the first time that this multi-vector system is triggered by the forager's exposure to the pheromone cue. Returning fan foragers will only complete the fan portion of their global path integrator when the pheromone column is not experienced. Fan foragers that were experimentally briefly exposed to the pheromone, even in isolation to all other column cues, shifted their headings and paths to a combination of their remaining fan vector and the column vector (the global vector). These directional and distance findings reveal that foragers are retrieving the column vector memory and integrating it with the remaining fan vector memory rather than merely reloading the column vector state. These findings show that the two-vector system operates independently of the global vector state as fan foragers exposed to the pheromone shift to the global vector even when ~2.5m of the fan vector distance remains. Additionally, we exclude use of the panorama views or other social cues of the column as a triggering cue to produce the vector switch. As a final finding, foragers show no evidence of using terrestrial cues to return to the column head and rely heavily on path integration while in the fan.

General discussion of results

First, we show that foragers returning in the fan rely heavily on their fan vector in order to return to the column head and show no evidence of orienting to either the column head or nest entrance using terrestrial cues or other cue sets after local displacement. Additionally, foragers collected from the column at the nest entrance (zero vector) and displaced near the column head exhibited backtracking behaviours, a backup mechanism expressed when terrestrial cues are uninformative and foragers have recently run off their path integrator (Wystrach et al. 2013; Freas et al. 2019c). Foragers collected in the fan navigated using the remaining fan vector when displaced either locally or distantly, orienting in the column head compass direction and exhibited pre-search path lengths that coincided (mean $\pm$ S.E., Nest 2: 2.59 ± 0.15 m and Nest 3: 1.40 ± 0.10 m) with the condition's mean vector distance to the column head (Nest 2: 2.5m and Nest 3: 1.5m) in line with previous work (Plowes et al. 2019). In contrast, when foragers collected from the fan were exposed to the column, both foragers' heading directions and path lengths changed. Headings shifted in the direction of the column vector, yet these shifts were incomplete and significantly differed from both fan collected control headings and column head collected forager headings.

Only during testing at Nest 3, with the smallest directional difference between the column vector and global vector (9°), did column head collected forager initial headings and column exposed forager initial headings not significantly differ, yet when foragers' pre-search paths were analysed beyond the first 30cm, these heading directions were significantly different. Additionally, column exposed foragers' pre-search paths at Nest 3 were significantly longer than both fan collected and column head collected foragers. These observed differences in forager path direction and length suggest that the observed headings were the result of the coupling of the column vector with the forager's remaining fan vector and not a reloading of the path integration state of the column vector after column exposure.

Furthermore, testing above the column indicated that the panorama along the foraging column does not induce this heading shift as fan collected foragers tested above the column still oriented in the direction of the fan vector. In contrast, column exposed foragers tested above the column did exhibit the heading shift to the global vector direction identical to previous conditions. Additionally, both exposure to conspecifics travelling in the column and access to the overhead celestial cues during column exposure were not critical to the observed heading changes, as foragers placed on the column under a blocking phial still exhibited the heading shift toward the global vector. Instead, exposure to the pheromone cue appears to mediate the recall of the column vector and its integration with the remaining fan vector, as fan foragers exposed to the pheromone in isolation to other column cues did shift their headings to the global vector.

When foraging in the fan, *V. pergandei* foragers appear to ignore their global vector and rely solely on the fan vector segment to return to the column head. These foragers will run the full vector distance before beginning to search for the column head while showing only a small directional bias in the nest compass direction (see Figure 5, 6; Plowes et al. 2019). This bias is only observed in some conditions while no bias is observed in others, suggesting an almost complete suppression of the global vector while in the fan. Yet, as soon as the forager reaches the pheromone trail the global vector becomes salient and is combined with the remaining fan vector. Under natural conditions this remaining fan vector would be near zero as foragers would have run off the fan vector while returning to the vicinity of the column head, but through experimental manipulation (Figure 3a; Figure 7a) we show that this remaining vector is integrated with the column vector once foragers reach the column rather than merely reloading the column vector state.

Local vector or multi-vector system

The two-vector system described in the current experiment could be argued to share some similarity to evidence of local vector use in solitary foraging ants (Collett et al. 1998). Here, a known visual panorama along a foraging route triggers the retrieval of a distance and directional memory to travel during the next route segment using the celestial compass and odometer but independent of the global vector and the foragers current path integration state (Collett and Collett 2015). This comparison would come with the clear difference that here the triggering memory retrieval cue is olfactory instead of visual. However, celestial compass and odometer dictated local vector segments triggered by familiar views remains controversial in the literature with early evidence for this type of segment memory also being explained by foragers setting travel directions through visual panorama guidance alone (Baddely et al. 2011, 2012; Lent 2013; Collett and Collett 2015; Hoinville and Wehner 2018; Webb 2019). Such debate only makes the current study's results all the more striking as this would be evidence of route segment memories directed by the celestial compass and odometer that would be presented without the terrestrial panorama also providing directional information through view matching. When the pheromone is presented alone within the phial, it likely provides the forager with no useful directional information and it is only through the cue's triggering of a vector segment memory that leads to the observed shifts.

Cue Integration

Given the pheromone cue presented in isolation is likely directionally uninformative, the cue instead appears to act as a context cue triggering memory retrieval of the column segment memory and subsequent integration between fan and column vector memories. As such, this would be an instance of cue integration both within the vector memory system as well as interaction between cues sets. During natural foraging, ant navigators often simultaneously acquire directional guidance from multiple information streams and are known to share and integrate both within and between different navigational systems and across sensory modalities (Wehner et al. 2016; Freas and Schultheiss 2018). Both within and between strategies, the available navigational information is typically redundant, with all cue sets in agreement. However, cues can be experimentally set in conflict to characterise how multiple processes are

engaged concurrently. Within the path integration system, multiple ant species have been shown use combinations of short-term and long-term vector memories to return to known resource sites and calibrate their path integration system when these memories deviate (Collett et al. 1999; Freas and Cheng 2018). Navigational systems can also communicate. When visual terrestrial cues and the path integrator conflict, foragers will weight these cues when orienting. Often one cue set will dominate (Collett et al. 1998; Narendra et al. 2007; Bregy et al. 2008), but foragers may also weight separate cue sets equally and choose a compromise direction (Collett 2012; Legge et al. 2014; Narendra et al. 2007; Freas and Cheng 2017).

Specifically exploring cross-modality cue interaction where one cue set acts as a context cue, similar to the current study, these interactions have also been shown in solitarily foraging ants. Context cue interactions occur between olfactory cues and the path integrator with *Cataglyphis fortis* foragers responding to the carbon dioxide plume of the nest entrance only when their path integrator was near zero (Buehlmann et al. 2012). However, the current study is the first time to our knowledge, that olfactory cues have been shown to mediate the current goal location of the path integrator. For a more complete review of cue interactions see Wehner et al. (2016).

Conclusions

In the current study, we found that *V. pergandei*'s column and fan foraging structure is underpinned by a two-vector path integration system mediated by exposure to the pheromone cues of the column. In this species vector segment memories are not controlled by exposure to well-known terrestrial cues along the route. Instead, the pheromone presented alone, led to foragers switching from using the fan vector to the global vector. Column exposed forager orientation to the global vector indicates that foragers retrieved and combined the column vector memory with their remaining fan vector instead of reloading the column vector state.

Compliance with Ethical Standards

Funding: This study was funded by a NSERC Discovery grant (#04133).

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Additionally, all testing conditions were non-invasive and all individuals were returned to the nest after testing.

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Tables

Table 1 Circular statistics for forager initial orientations in the local displacements around column head tests. Inbound foragers were collected from one of two locations before displacements, the column head (CH) or just before reaching the nest entrance (Nest). Foragers were displaced to one of five sites with the 0° site being raised ~15cm above the column.

Condition	n	Mean Vector	95% CI		Rayleigh Test		V-test		V-test	
			Minus	Plus	Z	p	Column Head	Column Vector	Column Head	Column Vector
							V	p	V	p
3.5m Displacements										
0° Site (Above Column)										
-Nest 1 (CH)	10	184°	166°	203°	8.2	<0.001	-0.90	1.00	0.90	<0.001
-Nest 4 (CH)	30	172°	162°	181°	24.0	<0.001	-0.89	1.00	0.89	<0.001
90° Site										
-Nest 1 (CH)	30	169°	148°	190°	11.1	<0.001	0.11	0.19	0.60	<0.001
-Nest 4 (CH)	30	171°	163°	180°	25.1	<0.001	0.14	0.15	0.90	<0.001
-Nest 1 (Nest)	30	337°	317°	357°	11.9	<0.001	-0.24	0.97	-0.58	1.00
-Nest 4 (Nest)	20	3°	350°	17°	15.1	<0.001	0.05	0.38	-0.87	1
180° Site										
-Nest 1 (CH)	10	180°	156°	203°	7.3	<0.001	0.85	<0.001	0.85	<0.001
-Nest 4 (CH)	30	173°	165°	181°	25.5	<0.001	0.92	<0.001	0.92	<0.001
270° Site										
-Nest 1 (CH)	30	195°	180°	211°	16.7	<0.001	0.29	0.06	0.72	<0.001
-Nest 4 (CH)	30	170°	155°	185°	17.2	<0.001	-0.14	0.85	0.74	<0.001
-Nest 1 (Nest)	30	120°	2°	38°	13.7	<0.001	-0.23	0.96	-0.64	1
-Nest 4 (Nest)	20	358°	343°	14°	13.7	<0.001	0.03	0.44	-0.83	1
1m Past Nest										
-Nest 1 (CH)	30	183°	170°	196°	8.2	<0.001	-0.81	1.00	0.81	<0.001
-Nest 4 (CH)	30	174°	160°	189°	17.8	<0.001	-0.77	1.00	0.77	<0.001

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Table 2 Circular statistics for forager headings in Experiments 2-6.

Condition	<i>n</i>	Mean Vector	95% CI		Rayleigh Test		V-tests							
			Minus	Plus	<i>Z</i>	<i>p</i>	True Nest		Fan Vector		Column Vector		Global Vector	
							<i>V</i>	<i>p</i>	<i>V</i>	<i>p</i>	<i>V</i>	<i>p</i>	<i>V</i>	<i>p</i>
Cross Fan Displacements														
<i>Nest 1</i>														
270° to 90° Cross Fan Control	30	258°	249°	267°	24.3	<0.001	-0.14	0.54	0.88	<0.001	0.19	0.07	0.38	0.001
90° to 270° Cross Fan Control	30	89°	65°	113°	9.2	<0.001	-0.12	0.81	0.55	<0.001	0.01	0.53	0.12	0.19
270° to 90° Column Exposure	30	199°	189°	209°	23.6	<0.001	0.75	<0.001	0.29	0.005	0.84	<0.001	0.88	<0.001
90° to 270° Column Exposure	30	158°	149°	168°	24.3	<0.001	0.74	<0.001	0.34	0.004	0.83	<0.001	0.89	<0.001
270° to 90° Fan Exposure	25	246°	234°	258°	19.2	<0.001	0.11	0.22	0.80	<0.001	0.36	0.01	0.58	<0.001
90° to 270° Fan Exposure	30	111°	96°	126°	17.8	<0.001	0.06	0.33	0.72	<0.001	0.28	0.02	0.47	<0.001
Distant Displacements														
<i>Nest 2 (90°)</i>														
Fan Control	21	98°	81°	115°	12.5	<0.001	-	-	0.77	<0.001	0.11	0.25	0.25	0.053
Column Head	24	177°	157°	196°	11.6	<0.001	-	-	0.04	0.40	0.70	<0.001	0.69	<0.001
Column Exposure	20	146°	133°	160°	14.9	<0.001	-	-	0.48	<0.001	0.72	<0.001	0.80	<0.001
<i>Nest 3 (270°)</i>														
Fan Control	30	267°	255°	279°	21.7	<0.001	-	-	0.85	<0.001	0.05	0.36	0.18	0.08
Column Head	30	187°	168°	205°	13.2	<0.001	-	-	0.08	0.281	0.84	<0.001	0.88	<0.001
20sec on Column	25	203°	194°	213°	20.8	<0.001	-	-	0.36	0.005	0.66	<0.001	0.66	<0.001
Forager Paths														
<i>Nest 1 (90°)</i>														
Fan Control-mean path	14	296°	290°	303°	13.5	<0.001	-	-	0.98	<0.001	0.23	0.116	0.66	<0.001
Fan Control-search onset	14	311°	304°	317°	13.5	<0.001	-	-	0.94	<0.001	0.46	0.007	0.82	<0.001
Column Head-mean path	15	10°	3°	17°	14.3	<0.001	-	-	0.22	0.119	0.92	<0.001	0.88	<0.001
Column Head-search onset	15	12°	7°	16°	14.7	<0.001	-	-	0.19	0.149	0.99	<0.001	0.88	<0.001
Column Exposure-mean path	15	337°	331°	344°	14.4	<0.001	-	-	0.70	<0.001	0.80	<0.001	0.97	<0.001
Column Exposure-search onset	15	343°	336°	350°	14.4	<0.001	-	-	0.63	<0.001	0.85	<0.001	0.98	<0.001
<i>Nest 3 (270°)</i>														
Fan Control-mean path	15	61°	55°	65°	14.6	<0.001	-	-	0.86	<0.001	0.49	0.003	0.59	<0.001
Fan Control-search onset	15	58°	50°	65°	14.2	<0.001	-	-	0.90	<0.001	0.52	0.002	0.62	<0.001
Column Head-mean path	15	2°	358°	6°	14.8	<0.001	-	-	0.04	0.41	0.99	<0.001	0.99	<0.001
Column Head-search onset	15	2°	358°	6°	14.7	<0.001	-	-	0.29	0.14	0.99	<0.001	0.99	<0.001
Column Exposure-mean path	15	27°	22°	33°	14.6	<0.001	-	-	0.46	0.006	0.87	<0.001	0.92	<0.001
Column Exposure-search onset	15	21°	16°	25°	14.8	<0.001	-	-	0.51	0.002	0.93	<0.001	0.96	<0.001
Column Panorama Exposure														
270° Above Column	29	258°	241°	274°	15.5	<0.001	0.15	0.12	0.71	<0.001	0.15	0.12	0.40	0.001
90° Above Column	30	96°	77°	114°	13.3	<0.001	0.06	0.31	0.66	<0.001	0.06	0.31	0.30	0.01
270° Column Exposure	30	204°	195°	212°	24.9	<0.001	0.84	<0.001	0.36	0.002	0.84	<0.001	0.91	<0.001
Column Head and Exposure	60	181°	174°	188°	48.1	<0.001	0.89	<0.001	-	-	0.89	<0.001	-	-
90° Column Exposure	30	169°	158°	179°	23.1	<0.001	0.86	<0.001	0.18	0.09	0.86	<0.001	0.87	<0.001
Pheromone Tests														
Fan Forager Control	31	94°	87°	101°	27.2	<0.001	-	-	0.93	<0.001	0.48	0.316	0.39	<0.001
20sec on Column Covered	30	146°	138°	153°	22.2	<0.001	-	-	0.53	<0.001	0.77	<0.001	0.90	<0.001
20sec Empty Phial	29	96°	88°	104°	25.9	<0.001	-	-	0.92	<0.001	0.10	0.225	0.42	<0.001
20sec Pheromone Phial	30	158°	147°	169°	24.8	<0.001	-	-	0.32	0.001	0.80	<0.001	0.86	<0.001

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Figures

Figure 1. Schematic diagram and circular distributions of the local displacements around column head tests. (a) Diagram of the column and fan foraging structure in *Veromessor pergandei* along with the testing procedure in the local displacement around the column head conditions. Foragers travel to and from the nest entrance using a pheromone-based column. At the end of this column, represented by the grey semi-circle, foragers fan out to collect food individually and then return to the column (black arrows). Foragers were collected from the column head and released at one of five local sites (grey arrows): 90°, 180°, 270°, 0° over column and 1m past sites. Circular distributions show individuals' initial orientations in 15° bins at 30cm at (b-f) Nest 1 and (g-k) Nest 4. The arrow in each distribution denotes the length and direction of the mean vector and the 95% CI is marked with the black line/circles. The grey triangle points in the column head direction, the white triangle points to the column vector direction, and the black triangle points to

833 the true nest direction. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.
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836 **Figure 2.** Schematic diagram and circular distributions of the local displacements with zero
837 vector foragers. (a) Returning column foragers were collected just before they entered the nest
838 and released at one of two sites: 90° or 270°. Circular distributions show individuals' initial
839 orientations in 15° bins at 30cm at (b,c) Nest 1 and (d,e) Nest 2. In each panel the direction for
840 the column vector was 180° and the direction predicted for backtracking was 0°. The arrow in
841 each distribution denotes the length and direction of the mean vector and the 95% CI is marked
842 with the black line/circles. In each distribution, the grey triangle points in the column head
843 direction, the white triangle points to the column vector direction, and the black triangle points to
844 the true nest direction. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

Figure 3. Schematic diagram and circular distributions of the cross-fan conditions. Diagrams of the collection and testing procedures in the (a) Cross Fan Controls and the (b) Column Exposure tests. Foragers were collected from the dark grey area of the foraging fan and displaced across the fan with or without exposure to the column cues. Circular distributions show individual initial orientation in 15° bins at 30cm in the (c) 270° to 90° Cross Fan Control, (d) 90° to 270° Cross Fan Control, (e) 270° to 90° Column Exposure, (f) 90° to 270° Column Exposure, (g) 270° to 90° Fan Exposure and (h) 90° to 270° Fan Exposure conditions. In each distribution, the white triangle denotes the fan vector direction (either 100° or 260°), the grey triangle denotes the column head direction (180°) and the black triangle denotes the true nest direction. The arrow in each distribution denotes the length and direction of the mean vector and the 95% CI is marked with the black line/circles. *n*, number of individuals; $\bar{\theta}$, mean vector; *r*, length of the mean vector.

Figure 4. Circular distributions of individual forager initial orientations during distant displacement experiments at (a-c) Nest 2 and (d-f) Nest 3. For each panel the column vector direction is set at 180° and marked with a grey triangle. The fan vector direction, 100° at Nest 2 and 260° at Nest 3 was marked with a white triangle. The global vector, 165° at Nest 2. and 189° at Nest 3 are marked with a black triangle. The arrow in each distribution denotes the length and direction of the mean vector and the 95% CI is marked with the black line/circles. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

Figure 5. Forager paths and circular distributions after distant displacement at Nest 1. Fan Control and Column Head foragers were collected and transferred to the distant site and tested. Column Exposure foragers were collected from the fan and released in the column for 20sec before recollection and testing. At Nest 1, Fan foragers were collected from the 2-3m and 70°-90° fan collection area, and displaced distantly to a (a) 5m × 4m grid where their paths were recorded. For each condition, the (b) mean path direction and (b) direction at the onset of search were recorded from the release point. In each distribution, the 95% CI is marked with the black line/circles with the centre line denoting the direction of the mean vector. The light grey triangle shows the fan to column head compass direction, the black triangle shows the fan to nest compass direction and the dark grey triangle shows the column-head-to-nest compass direction. The number of individuals in each condition is stated in parentheses.

Figure 6. Forager paths and circular distributions after distant displacement at Nest 3. Fan Control and Column Head foragers were collected and transferred to the distant site and tested. Column Exposure foragers were collected from the fan and released in the column for 20sec before recollection and testing. At Nest 3, Fan foragers were collected from the 1-2m and 270°-290° fan collection area, and displaced distantly to a (a) 3m × 8m grid where their paths were recorded. For each condition, the (b) mean path direction and (b) direction at the onset of search were recorded from the release point. In each distribution, the 95% CI is marked with the black line/circles with the centre line denoting the direction of the mean vector. The light grey triangle shows the fan to column head compass direction, the black triangle shows the fan to nest compass direction and the dark grey triangle shows the column head-to-nest compass direction. The number of individuals in each condition is stated in parentheses.

Figure 7. Schematic diagram and circular distributions of the Column Panorama Exposure conditions. Diagrams of the collection and testing procedures in the (a) Above Column controls and the (b) Column Exposure tests. Fan foragers were collected from the fan collection area (dark grey) while column head foragers were collected from the end of the column. Circular distributions show individual initial orientations in 15° bins at 30cm in the (c) 270° Above Column, (d) 90° Above Column, (e) 270° Column Exposure, (f) Column Head and Exposure and (g) 90° Column Exposure conditions. In each distribution, the white triangle denotes the fan vector direction, the grey triangle denotes the column head direction and the black triangle denotes the true nest direction. The arrow in each distribution denotes the length and direction of the mean vector and the 95% CI is marked with the black line/circles. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.

Figure 8. Circular distributions of individual forager initial orientations during the phial conditions at Nest 1. Fan foragers were collected and either (a) immediately transferred and tested distantly, (b) released onto the foraging column under the phial, (c) held in the empty phial for 20 sec before testing, or (d) held for 20sec in a phial containing dirt from the column and then tested. For each panel the column vector direction is set at 180° and marked with a grey triangle. The fan vector direction, 100° at Nest 2 and 260° at Nest 3 is marked with a white triangle. The global vector, 165° at Nest 2. and 189° at Nest 3 are marked with a black triangle. The arrow in each distribution denotes the length and direction of the mean vector and the 95% CI is marked with the black line/circles. *n*, number of individuals; *Ø*, mean vector; *r*, length of the mean vector.

Supplemental Figure 1. Panoramic 360° photos of the nest site, the column head site and the distant testing sites for each nest. Each photo is positioned with the column to nest compass direction centred. The arrow denotes the true nest direction from each site.

Supplemental Figure 2. Circular distributions of initial headings in the Goniometer Control conditions. Initial headings of ants collected just before entering the nest. Distributions show initial orientation in 15° bins at 30cm. (a) The first forty foragers were tested pre-goniometer rotation. (b) The second group of foragers was tested after the goniometer was rotated in place 180°. The arrow in each distribution denotes the length and direction of the mean vector and the 95% CI is marked with the black line/circles. In each circular distribution the true nest direction is marked with a black triangle and the white triangle denotes the column vector direction. n , number of individuals; $\bar{\theta}$, mean vector; r , length of the mean vector.