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The role of attractive and repellent scene memories in ant homing (*Myrmecia croslandi*)

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Abstract

Solitary foraging ants rely on vision when travelling along routes and when pinpointing their nest. We tethered foragers of *Myrmecia croslandi* on a trackball and recorded their intended movements when the trackball was located on their normal foraging corridor (on-route), above their nest and at a location several meters away where they have never been before (off-route). We find that at on- and off-route locations, most ants walk in the nest or foraging direction and continue to do so for tens of metres in a straight line. In contrast, above the nest, ants walk in random directions and change walking direction frequently. In addition, the walking direction of ants above the nest oscillates at a fine scale, reflecting search movements that are absent from the paths of ants at the other locations. An agent-based simulation shows that the behaviour of ants at all three locations can be explained by the integration of attractive and repellent views directed towards or away from the nest, respectively. Ants are likely to acquire such views via systematic scanning movements during their learning walks. The model predicts that ants placed in a completely unfamiliar environment should behave as if at the nest, which our subsequent experiments confirmed. We conclude first, that the ants' behaviour at release sites is exclusively driven by what they currently see and not by information on expected outcomes of their behaviour. Second, that navigating ants might continuously integrate attractive and repellent visual memories. We discuss the benefits of such a procedure.

250 words

Introduction

Navigation on a local, in contrast to a global, scale involves travelling along routes and pinpointing places (e.g. Zeil 2012). Much evidence has accumulated to show that ants form visual memories of how the scene looks along routes (e.g. Wehner et al., 1996; Wystrach et al., 2011; Mangan and Webb, 2012) and that alignment matching (Zeil et al., 2003; Collett et al., 2013) between memorized and currently experienced views provides robust information on heading direction (Graham and Cheng, 2009; Baddeley et al., 2011, 2012; Narendra et al., 2013; Zeil et al., 2014). Heading direction can be recovered, even from locations at some distance from familiar locations, by detecting the minimum of the rotational image difference function resulting from a comparison between current and memorised views (rotIDF, Zeil et al., 2003, Stürzl and Zeil, 2007, Philippides et al., 2011; Narendra et al., 2013; Stürzl et al., 2015). This minimum provides a measure of familiarity in addition to a heading direction (Baddeley et al., 2011, 2012; Graham et al., 2010).

Before becoming foragers, ants perform a series of learning walks around the nest during which they alternate between turning to look in the nest direction (Müller and Wehner, 2010; Fleischmann et al., 2016, 2017, 2018a,b) and in directions away from the nest from different compass directions (Jayatilaka et al., 2018; reviewed in Zeil and Fleischmann, 2019). It is attractive to assume that the ants store snapshots during these turns whenever they are aligned parallel to the home vector, that is, when they are facing toward or away from the nest direction (Wehner and Müller, 2010; Graham et al., 2010; Jayatilaka et al., 2018), as this is theoretically sufficient for returning ants to align with and walk into the direction of the most familiar of nest-directed snapshots in order to pinpoint the nest (Graham et al., 2010; Baddeley et al., 2012; Wystrach et al,. 2013).

Such visual ‘alignment matching’ (Collett et al., 2013) explains well how ants recover the correct direction when on their familiar route (Wystrach et al., 2011b; Wystrach et al., 2012; Baddeley et al., 2012; Kodzhabashev and Mangan, 2015). Moreover, nest-directed views acquired during learning walks (reviewed in Collett and Zeil, 2018; Zeil and Fleischmann, 2019) can also provide guidance from locations that are unfamiliar to ants and that can be 10-15m away from the nest in open forest habitats (Narendra et al., 2013; Stürzl et al., 2015), although

the initial movements of released ants may not be directed toward the nest (Zeil et al., 2014), but toward a familiar route (Collett et al., 2007; Wystrach et al., 2012).

Overall, this line of work has led to the suggestion that visually navigating insects would only need 'procedural knowledge' about knowing where to go rather than requiring a more sophisticated representation of their spatial environment that would allow them 'to know where they are' (Collett et al., 2002; Wehner et al., 2006; Cheung et al., 2014; Graham and Philippides, 2017).

To test this directly, we positioned ants that we had tethered over a trackball at different locations in their natural foraging environment, including above their nest, and recorded their intended direction and distance of movement. Ants mounted on the ball were well oriented towards the nest at both on and off route locations, but displayed a search pattern when above the nest, as if they knew they were at the nest, implying a sort of positional rather than just procedural knowledge. Using a simple agent-based-simulation we show, however, that these results can be more parsimoniously explained by alignment matching involving continuous integration of attractive and repellent visual memories, acquired when facing respectively towards and away from the nest during learning walks.

Materials and Methods

Ants and experimental site

We worked with foragers of the Australian Jack Jumper ant *Myrmecia croslandi* from a nest in the Australian National University's campus field station (-35° 16' 49.87''S and 149° 06' 43.74''E). The ants are day-active, visually navigating solitary foragers that hunt for insects on the ground at up to 4 m distance from the nest and on trees, about 12 m away from the nest where they also feed on sugar secretions of plant-sucking insects (see centre panel top row, Fig. 2). For details of the foraging ecology and the navigational abilities of these ants see Jayatilaka et al. (2011, 2014), Narendra et al. (2013) and Zeil et al. (2014). During February to March 2017 and December 2017 to March 2018, between 9:00 and 15:00, we caught foraging ants either at their foraging trees about 12 m from the nest in a 'full vector' state (FV, n=10) or at the nest in 'zero vector' state (ZV, n=18), offered them sugar water solution to feed on

before immobilizing them on ice for up to 15 min and tethering them to a metal pin by their mesonotum (thorax) using Bondic liquid plastic welder (Biochem Solutions, Ellerslie, New Zealand). The ants were placed on an air-cushioned light-weight, 5 cm diameter track ball (Fig. 1A) on which they were free to rotate around the yaw axis but that allowed us to record their intended translational movements as described in detail by Dahmen et al. (2017). We placed the trackball contraption with a tethered ant at each of three locations in a random order (Fig. 2, top row centre panel): 6.5 m west of the nest where none of the ants were likely to have been before (Off-route), 6.5m south of the nest, half-way along their normal foraging route towards trees (On-route), and directly above the nest (Nest).

We recorded the intended movement directions and distances on the trackball at each displacement for up to 10 minutes, before shifting the track ball contraption together with the tethered ant to the next location. Ants were carefully un-tethered and released close to the nest following the three displacements.

To demonstrate the foraging patterns of ants at this nest and the full range of learning walks, we show the paths of foraging ants, ants that performed learning walks and ants that were released after contributing to unrelated experiments that were recorded with Differential GPS over two years (Fig. 2, top row centre panel and Fig. S1; for details see Narendra et al., 2013). In brief, coloured flag pins were placed on the ground approximately 20 cm behind a walking ant at fairly regular intervals, carefully avoiding disturbing her progress. The resulting pin trail was subsequently followed with the rover antenna of a Differential GPS system, recording the position with an accuracy of better than 10 cm.

Data analysis

We recorded trackball rotations due to the intended translation of the ants at 275 fps, which reflect the direction and speed of the ants' intended movements. We present the reconstructed paths, final bearings, changes in walking direction and path lengths for the first 5 min of recordings at the three displacement locations. With the exception of one ant at the off-route location, all ants reached this criterion. We used the Matlab (MathWorks, Natick, MA, USA) circular statistics toolbox (by Philipp Berens) to perform Rayleigh's test for non-uniformity on directional data and Wilcoxon Rank Sum tests on differences between displacement locations using the ranksum function in Matlab. For comparisons between all

three locations we applied a Bonferroni correction with a resulting critical value for individual tests of p=0.0167.

Agent-based modelling

Reconstructed world and ant views: We rendered panoramic views within a 3D model of the ants' natural environments that was previously reconstructed at the ANU Campus Field Station using a laser scanner and camera-based methods (Stürzl et al., 2015). We down-sampled the rendered views to 360×180 pixels, that is, 1°/pixel resolution to roughly match the resolution of the ants' compound eyes. Note that the 3D model was obtained 3 years before the treadmill experiments were conducted, so that there will be some changes to the landmark panorama, in particular involving the canopy, while all the major geometric relationships of dominant visual features such as trees will have remained the same.

Memorised views and current familiarity: The agent is assumed to have stored a collection of memorised views around the nest during learning walks and along their normal foraging route (Fig. 1B). During tests, the agent is computing a value of visual familiarity at each time step by comparing the current view to its memory bank. This is achieved by calculating the global root mean squared pixel difference (Zeil et al., 2003) between the current view and each of the views in the memory bank, and keeping the value of the lowest mismatch, as is typically done in models and studies of ant navigation (Wystrach et al., 2011b; Wystrach et al., 2012; Baddeley et al., 2011, 2012; Philippides et al., 2011; Narendra et al., 2013, Zeil et al., 2014, Stürzl et al., 2015). Because high mismatch values indicate a large discrepancy between the current and a memorized view, the value indicates the current unfamiliarity score rather than a familiarity score. Note that in the insect brain, the activity of the mushroom body output neurons (MBON) also correlate with unfamiliarity rather than familiarity (Owald et al., 2015; Felsenberg et al., 2018). Importantly, views in this model are not rotated, but compared only at the facing direction of the current and memorized views. That is, the agent does not need to stop and scan because only one view is compared for each step.

Combining attractive and repellent visual memories: The novel aspect of this current model is that the agent is assumed to have two independent memory banks (Fig. 1B-D): one containing attractive views and one containing repellent views. Both memory banks contain

views experienced during learning and foraging walks; the attractive memory bank containing views that are assumed to have been memorised when the ants were oriented toward the nest and the repellent memory bank those that have been memorised while looking away from the nest. This is motivated by the very regular scanning movements of ants during their learning walks where they alternate looking towards the nest and away from the nest direction (Jayatilaka et al., 2018; Zeil and Fleischmann, 2019). For simplicity, learning walk views were assumed to have been acquired within a 1 m radius around the nest and we chose a 10 m long route, corresponding roughly with the foraging corridor of this particular nest (see Fig. 2, top centre panel). Both nest-directed (attractive) learning walk views and views away from the nest (repellent) were taken from positions along a spiral rather than a circle around the nest (Fig. 1B), to mimic the fact that successive learning walk loops reach increasing distances from the nest (e.g. Fleischmann et al., 2016; Jayatilaka et al., 2018) and to ensure that results at the nest were not dependent on having views memorised at the exact same distance from the nest. We also included in the attractive memory bank views that foragers experience when travelling back to the nest along their normal foraging corridor (Fig. 1B).

Modelling procedure: At each time step, the agent computes two values of unfamiliarity: one by comparing the current view to the attractive memory bank and one by comparing the same current view to the repellent memory bank (Fig. 1C & D). These two unfamiliarity values are assumed to have an antagonistic effect on the agent's behaviour by turning it towards attractive and away from repellent stimuli with the balance between the two drives determining the agent's turning direction. We modelled this by a simple subtraction resulting in a raw overall drive

Raw overall drive = (attractive unfamiliarity value – repellent unfamiliarity value) / 0.2 (1)

We normalised the value of this drive by using always the same value (0.2 in our world), corresponding roughly to the unfamiliarity score obtained between views from locations in the virtual world that are far apart, so that *Raw overall drive* will be contained between -0.5 and 0.5. A negative value thus indicates that 'attractive unfamiliarity' < 'repellent unfamiliarity'. A positive value indicates that 'attractive unfamiliarity' > 'repellent unfamiliarity'. We then transform *Raw overall drive* into an *Overall drive* with values ranging from 0 to 1 using a simple sigmoid function:

Overall drive = Sigmoid (Raw overall drive) (2)

As a result, the *Overall drive* tends towards 0 if 'attractive unfamiliarity' < 'repellent unfamiliarity', towards 1 if 'attractive unfamiliarity' > 'repellent unfamiliarity' and is 0.5 if 'attractive unfamiliarity' = 'repellent unfamiliarity'. In other words, a low score indicates that the current view matches a view in the attractive memory bank better than in the repellent memory bank and a high score indicates that the current view matches a view in the repellent memory bank better than in the attractive memory bank (Fig. 1C).

To drive the agent, we used a similar approach to Kodzhabashev and Mangan (2015). The agent is a simple dot in space (x,y) with a current heading (theta). The agent has a continuously running oscillator alternating between left mode and right mode, which controls the current turning direction. For simplicity, we modelled this by simply alternating the turning direction at each time step (Left-Right-Left-Right) as in Kodzhabashev and Mangan (2015). The resulting paths typically show sharp zigzags, however it is worth noting that alternating turning direction every 4^{th} step produces smoother oscillations that better resemble real ant paths (Fig. 1E).

Turn direction is thus purely controlled by the oscillator, however, the *Turn amplitude* is directly dependent on the current *Overall drive* (see previous section), that is, on the current view familiarities.

Turn amplitude (deg) = gain × Overall drive (3)

We use a single parameter (gain) to convert the *Overall drive* (between 0 and 1) into the angular value for the turn amplitude. We simply used gain = 180, so that the turning amplitude would vary between 0 degrees (if *Overall drive* = 0) and 180 degrees (if *Overall drive* = 1), with 90 degrees if *Overall drive* = 0.5, that is if attractive and repellent unfamiliarity values are equal.

Across time steps (n), the agent orientation (theta) will thus alternate between left and right turns ($(-1)^n$), with each turn varying between 0 and 180 degrees.

$$Theta(n+1) = Theta(n) + (Turn\ amplitude \times (-1)^n) + noise$$

To ensure that the agent is robust against the intrinsic noise of the real world, we added noise at each time step, as a random angular value drawn from a Gaussian distribution (mu=0; std=10 degrees).

Agent on a fictive tread-mill: We simulated agent behaviour on a fictive treadmill by simply preventing forward motion. That is, at each time step we assumed that the agent (1) obtains the current view and computes its *Overall drive* (Eqn 1 & 2); (2) turns on the spot with turn direction determined by the state of the oscillator and turn amplitude by Eqn 3 & 4. Since the location at which the agent is standing does not change, the view perceived at each time step only varies depending on the agent's current orientation. The agent on the tread mill was tested at different release locations and we recorded the resulting behaviour.

Using attractive visual memories only: We also tested the agent using the attractive memory bank only. In that case

Raw overall drive = attractive unfamiliarity/0.2 - 0.5.

Given that attractive unfamiliarity is always positive, we removed 0.5 during normalisation to centre the *Raw overall drive* on 0, ranging roughly from -0.5 to 0.5 in the same way as when combining attractive and repellent memories. We then used the same sigmoid function to obtain an *Overall drive* between 0 and 1 (Eqn 2).

Results

Myrmecia ants released on the tread-mill

Irrespective of whether they were caught in a zero-vector state (ZV) or a full-vector state (FV), tethered ants behaved differently when placed 6.5 m west of the nest (off-route, Fig. 2, top left panel), 6.5 m south of the nest (on-route, Fig. 2, bottom middle panel) or over the nest (Nest, Fig. 2, top right panel).

In the off-route and on-route locations, most intended paths of both ZV and FV ants were goal directed either to the nest or to the individuals' specific foraging trees (see inset circular histograms in Fig. 2). This is to be expected for *M. croslandi* foragers, which ignore path integration information in the FV state as long as the landmark panorama provides

navigational information (see Narendra et al., 2013; Zeil et al., 2014). The paths tended to be straight (see Fig. 3D). In contrast, over the nest, ZV ants moved in random directions, while FV ants tended to move roughly along the home vector direction to the north (at 90^{o} Fig 2, black tracks, top right panel, see also Fig. 3B). Both ZV and FV ants at the nest changed their walking direction frequently. Inset histograms show that most tethered ants over the nest ended up after 5 minutes at final virtual distances less than 10 m from the nest (median 6.07 m), while at the on-route location, most ants reached much larger virtual distances (median 12.61 m) in the same amount of time (Wilcoxson Rank Sum test: nest vs on-route distances are different: $p = 0.0045$; $z = 2.8378$. See Fig. S2A). The median distances reached at the off-route location are not larger than the ones at the nest (median 6.2 m), owing to a conspicuous peak at small distances contributed by ants that were lost at this location.

The behaviour of ants at the off-route location is interesting primarily because most ants are home directed despite it being unlikely that they have ever been to this location before (see inset circular histogram, Fig. 2, top row left panel). A heat map of the foraging movements of 124 ants from this nest that had been DGPS-tracked on their outward foraging trips over two years shows that no ant had moved off-route of the nest for more than a few meters (Fig. 2, top row middle panel). Some of the tethered ants appear to have headed towards their foraging trees or the foraging corridor in south-easterly direction, however, when we track ants that were released just north of the off-route location, many initially for 2 m or so do walk in a south-easterly direction before turning east toward the nest (Fig. 2, bottom row, left panel). Tethered ants at the off-route location must therefore get their bearing by comparing what they currently see with nest-directed views they are likely to have gathered during their learning walks, which can extend up to 4 m from the nest (Fig. 2, bottom right panel. See also Jayatilaka et al., 2018).

Both FV and ZV ants at the on-route location decided to move either back toward the nest or south toward their foraging trees (Fig. 2, bottom row, centre panel). Otherwise, they moved in a similar way than when at the off-route location. Most of them moved fast, straight and for distances far exceeding those needed to reach the nest or the trees.

The most conspicuous feature of paths at the nest location is the fact that the initial walking direction of ZV ants is random, while those of FV ants is in the general home vector direction (north) and that both ZV and FV ants change walking direction frequently.

We quantify these differences between locations in three ways in Fig. 3, considering final bearings, the relationship between path length and distance reached and changes in walking direction. Fig. 3A shows the initial paths of ants at the three locations in more detail to emphasize the different behaviours and to highlight the additional fact that paths are fairly smooth at the off-route and on-route locations, but show a distinct sinusoidal oscillation at the nest location. With the exception of the bearings of ZV ants at the nest (Fig. 3C right panel) and those of FV ants after 5 min at the off-route and the nest location (Fig. 3C left and right panel) the virtual bearings of ants after 5 minutes or at 5 m distance from the start are all significantly different from uniform distributions, both for ZV (Fig. 3B) and FV ants (Fig. 3C). While the distributions are unimodal for the off-route and nest location (see insets Fig. 3B and C for circular statistics), they are clearly bimodal at the on-route location.

One measure of the straightness of paths is the way in which the straight-line distance from the start depends on path length (Fig. 3D), with straight paths without changes in direction lying close to the line of equality. After 5 minutes, the distribution of the ratios of final distance to final path length differs between the sites (see insets in Fig. 3D and Fig. S2B) with the on-route paths being significantly straighter with a median ratio of distance over path length of 0.83, compared with 0.62 at the off-route location and of 0.45 at the nest location (Wilcoxon Rank Sum test at 5% significance level: On-route *vs* off-route: p=0.0110; on-route *vs* nest: $p=8.4992e^{-4}$; off-route *vs* nest: p = 0.6. See Fig. S2B).

Finally, the behaviour of ants at the three sites also differs on a finer scale: the distribution of changes in path direction is much broader at the nest site, compared to the off-route and on-route location (Fig. 3E) reflecting the conspicuous oscillations of ant paths over the nest (see right panel, Fig. 3A). Note that these distributions have very long tails due to spikes of very high angular velocities which may be artefacts of trackball rotations when the ants are moving very slowly (see time series in Fig. 4). To test whether changes in path direction are indeed systematically larger at the nest location, we calculated the means of their absolute values at 11fps over the first 5 min of walking for each ant and compared their distributions, both for angular velocities smaller than 200^{o}/s (insets Fig. 3E and Fig. S2C) and for all angular velocities (Fig. S2D). Below 200^{o}/s, nest paths are indeed wigglier compared to on-route paths (Fig. S2C, Wilcoxon Rank Sum test: nest *vs* on-route $p=2.56e^{-4}$, z=-3.6563), with the difference between nest and off-route location just failing to reach significance (nest *vs* off-route p=0.019, z=-

2.3458). Considering the whole range of angular velocities (Fig. S2D) there is no difference between nest and the other locations, mainly because of high angular velocities exhibited by ants at all sites.

We note that many ants at various times during the first 5 minutes on the trackball over the nest show very regular path oscillations as documented in Fig. 3A and for three examples in Fig. 4 (red traces). The distribution and the time course of changes in path direction over the nest are different from those exhibited by the same ants at the on-route location (shown in blue in Fig. 4). Regular and sustained path oscillations lead to periodicities in the auto-correlation function of changes in path direction and can be detected in 13 out of 25 cases of ants participating in all three locations (blue traces in Fig. S3), compared to 4/25 at the off-route location (red traces in Fig. S3) and 1/25 at the on-route location (green traces in Fig. S3). We add the caveat that the statistics of path properties are unlikely to be stationary during an experiment and that this particular aspect of ant behaviour will require future attention.

Agent-based modelling

To model the agent on a fictive tread-mill, we simply prevented it from stepping forward, so that views were always perceived from the same spot, and where rotated according to the agent's current facing direction. We released the agent at four locations.

When tested close to the beginning of the homing route (on-route RP), the agent oriented mostly in the correct direction, that is, along the route towards the nest (blue paths in Fig. 5A). This is because the overall drive is close to 0 while facing in this direction (the attractive unfamiliarity is very low and the repellent unfamiliarity is high (Fig. 1C) yielding very small turns (Fig. 5B & C). Note that if the agent happened to face in the opposite direction (due to noise), the overall drive would strongly increase and thus trigger a large turn.

When released away from the route (off-route RP), the agent also favoured one direction indicating that this direction provided a smaller overall drive (yellow paths in Fig. 5A). This is an indication that the view at the off-route RP and nest-directed learning walk views are most

335 familiar because their comparison produces a detectable minimum of the rotIDF and that the
336 agent thus favours a direction roughly pointing towards the nest.

337 When released on top of the nest (nest RP), the agent produced convoluted paths with no
338 preferred directions (red paths in Fig. 5A). This is due to the rather uniform distribution of
339 visual familiarities across directions (see Fig. 1C). At a more local scale, the paths show much
340 larger turn amplitudes than at the on-route or off-route RPs (Fig. 5B & C). This is because at
341 the nest location, attractive and repellent memorised views provide a roughly equal match
342 whatever the current facing direction, resulting in an overall drive around 0.5, thus yielding
343 turns that are larger than when attractive and repellent memories match best for different
344 directions (see Fig. 1C).

345 When released at a distant unfamiliar location (distant RP), the agent displayed equally large
346 turn amplitudes as at the nest (marked in black in Fig. 5A-C) because, as for the nest location,
347 both the attractive and the repellent memory bank provide roughly equal unfamiliarity
348 values, thus resulting in an average overall drive around 0.5.

349 In contrast, when using the attractive memory bank only, turn amplitudes were large at the
350 distant unfamiliar location (black) but comparatively low at the nest (red, right column, Fig.
351 5). This is simply because the unfamiliarity value is high in the unfamiliar location (yielding a
352 strong directional drive and thus large turns), and low at the nest due to the good match with
353 learning walks views (yielding a low directional drive and thus small turns).

354

355 *Testing model-predictions with Myrmecia*

356 Motivated by the different simulation results when using 'attractive only' and
357 'attractive/repellent' memory banks as well as by the rather counter-intuitive outcome that
358 the use of 'attractive/repellent' memories predicts a similar behaviour at the familiar nest
359 location and at a completely unfamiliar location, we released *Myrmecia* ants mounted on the
360 trackball both at the nest and at a distant location about 50 m south-west of the nest. The
361 location was far beyond the ants' foraging trees and thus was likely to be completely
362 unfamiliar to the ants. Strikingly, ants at this distant release location behaved in a similar way
363 as at the nest, both in terms of the ratio between the distance reached after 5 minutes and

the path length (see box plot insets in Fig. 6A centre panels, Wilcoxon Rank Sum test unfamiliar *vs* nest location: p=0.7984, ranksum=71) and in terms of the mean absolute changes in walking direction (see box plot insets in Fig. 6A right panels, Wilcoxon Rank Sum test unfamiliar *vs* nest location: p=0.9591, ranksum=67). The ants at both the unfamiliar and the nest site also displayed the characteristic path oscillations we observed at the nest in our previous experiments (Fig. 6A and B, compare with Fig. 4), as predicted by the attractive/repellent model.

Discussion

Our behavioural experiments revealed three fundamental properties of visual navigation in ants that could only be uncovered using the trackball method. First, we determine that whether on-route or off-route, several metres away from the nest, ants can recover the goal direction without the need to physically move and to sample neighbouring locations. Second, we find no evidence that they 'expect' outcomes from their behaviour, such as a changing visual scene or increasing certainty about the location of the nest. *M. croslandi* ants show no evidence of monitoring the distance that separates them from the goal, unlike for instance ants that rely strongly on path integration (Dahmen et al., 2017). Third, ants behave differently when positioned above the nest, by following random heading directions and frequently changing their walking direction. These are the characteristics of search behaviour and thus could be interpreted as indicating that ants 'know' that they are at the nest, as if they possessed location information. However, our simulation results demonstrate that the nest-specific behaviour of ants can be parsimoniously explained by the density of attractive, nest-directed, and repellent views away from the nest that at least *M. croslandi* ants are likely to acquire in the course of systematic scanning movements during their learning walks (e.g. Jayatilaka et al., 2018). Our simulation also confirms that the same parsimonious mechanism can recover a correct direction from on- and off-route locations, as previous modelling has indicated (Baddeley et al., 2011, 2012; Narendra et al., 2013; Wystrach et al., 2013; Kodzhabashev and Mangan, 2015).

Alignment matching and visual memories

Current thinking holds that ants during their learning walks learn nest-directed views (*Cataglyphis sp*: Fleischmann et al., 2016, 2017, 2018a,b; *Ocymyrmex robustior*: Müller and Wehner, 2010; *Melophorus bagoti*: Wehner et al., 2004; Muser et al., 2005) and possibly both nest-directed views and views pointing away from the nest (*Myrmecia croslandi*: Jayatilaka et al., 2018; Zeil and Fleischmann, 2019). In addition, they memorize the views they experience along routes as they go back and forth on foraging excursions (Wehner et al., 1996; Mangan and Webb, 2012; Kohler and Wehner, 2005; Wystrach et al., 2010; Freas and Specht, 2019).

When using their visual memories to navigate, the currently perceived panorama provides a heading direction if the comparison between memorised views and the current view generates a detectable minimum of the rotational image difference function (see Narendra et al., 2013). This is a basic measure of familiarity and at any location, the direction presenting the most familiar view would provide the deepest (lowest) minimum. At both the on-route and off-route location ants on the trackball were free to scan the panorama and detect the direction of any present minima. Our results show that they were successfully able to recover the goal direction by doing so (Fig. 2). On route, some ants headed to the nest while others aimed at their foraging trees, reflecting their motivation to home or to forage.

While the directedness of ants at the on-route site would have been supported by both learning walk views and views learnt along the route, their directedness at the off-route (west) location depends on their detecting a higher similarity with learning walk views directed at the nest from the west compared to all other nest-directed views. As shown here and before (Narendra et al., 2013; Zeil et al., 2014; Stürzl et al., 2015), this is possible up to 10-15m distance from the nest in the open woodland habitats of *Myrmecia* ants, provided ants have acquired such nest-directed views about 1 to 5 metres away from the nest (see Fig. 2B, bottom right).

When released at the nest, ants behaved differently. They walked in various directions and displayed larger turns that regularly alternated between left and right, resulting in sinusoidal paths. So are nest views special?

As far as navigational information is concerned, the situation at the nest is indeed different compared with both on- and off-route sites. During their learning walks ants will have

encountered a dense set of views at different distances and compass bearings around the nest, each potentially tagged with the nest direction through path integration (Müller and Wehner, 2010; Graham et al., 2010; Baddeley et al., 2012; Fleischmann et al., 2018a; Jayatilaka et al., 2018; Zeil and Fleischmann, 2019). In contrast to other locations, tethered ants placed above the nest location thus will encounter attractive familiar views (or deep rotIDF minima) in many compass directions, which might explain why they initially walked in various directions at this location.

The high amplitude oscillation displayed by ants at the nest location, however, is puzzling. Previous models suggest that experiencing a familiar (attractive) view should inhibit turns and favour forward motion (Zeil, 2012; Möller, 2012; Baddeley et al., 2011, 2012; Wystrach et al., 2013; Kodzhabashev and Mangan, 2015; Ardin et al., 2016), which is here clearly not the case. The behaviour of tethered ants on top of the nest can be interpreted as search for the nest entrance, which in ants relying on path integration is characterized by frequent changes in path direction and a systematic pattern of increasing loops around the expected location of the goal (e.g. Schultheiss et al., 2015). To our knowledge, however, no analysis of the fine-scale changes in orientation of searching ants – as we observed them here - has been done to date.

Previous work has suggested that the recognition of views memorised at the nest may trigger specific behaviours when subsequently released in unfamiliar locations (Wystrach et al., 2013). This interpretation may suggest positional knowledge, or at least that views close to the nest are categorised separately from route views during learning and being treated differently when recognised. In the following we discuss the results of our simulation that suggest a parsimonious and unifying explanation for view-based route guidance, pinpointing goals and the current observation of high amplitude oscillation at the nest without the need to invoke positional knowledge or the need for a 'trigger' of search behaviour. Our agent-based modelling exhibits the same pattern of fine-scale oscillations, including overall changes in path direction, but only if we assume that the agent operates with both attractive and repellent memory banks.

Continuously integrating attractive and repellent views

Our model was developed quite independently to explain other recently observed phenomena, such as how ants manage to use views for guidance while walking backward and thus facing in the anti-nest direction (Schwarz et al., under review); or how ants learn to detour areas along their route associated with an aversive experience (Wystrach et al., 2019). Interestingly, this new model happens to also capture the current results remarkably well. The model is based on two assumptions: (1) that ants store both attractive and repellent views during their learning walks as suggested by Jayatilaka et al. (2018), and (2) that guidance involves an oscillator resulting in a continuous alternation between left and right turns (Namiki and Kanzaki, 2016; Kodzhabashev and Mangan, 2015; Wystrach et al., 2016). The model assumes no positional knowledge whatsoever, only procedural knowledge.

Several pieces of evidence suggest that insects possess an intrinsic oscillator triggering alternatively left and right body rotations, the amplitude of which can be modulated by the stimuli perceived (Namiki and Kanzaki, 2016; Lent et al., 2013; Wystrach et al., 2016). Such a control of oscillations can provide guidance along odour plumes (Namiki and Kanzaki, 2016) and odour gradients (Wystrach et al., 2016), support visual route following (Kodzhabashev and Mangan, 2015) and greatly facilitates the integration of different sources of stimulation (Wystrach et al., 2016). In the case of visual route following, the amplitude of the oscillations needs to be simply modulated by the familiarity of the currently perceived view. The suggestion is that familiar views trigger small turns whereas unfamiliar views trigger large turns, and that the direction of the turn is dependent on the current state of the oscillator. Because views are assumed to be memorized while moving along the route, during route recapitulation visual familiarity is higher when facing in the correct route direction. This model is sufficient for an agent to recapitulate a route in naturalistic environments (Kodzhabashev and Mangan, 2015). However, when released at the nest, this model does not predict large amplitude oscillations such as the ones we observed here in ants. On the contrary, because of the high familiarity experienced at the nest, which results from the collection of nest-oriented views acquired during learning walks, the model predicts an inhibition of the oscillations whatever the current facing direction (see Fig 5, right column).

The visual memories used by insect navigators are likely stored in the mushroom bodies (Webb and Wystrach, 2016), but current models assume only the existence of attractive memories (Möller, 2012; Baddeley et al., 2011, 2012; Wystrach et al., 2013; Kodzhabashev

484 and Mangan, 2015; Ardin et al., 2016). Here we incorporated into the model the recent
485 suggestion that ants store both attractive and repellent views, mimicking the so-called
486 'appetitive/aversive' output pathways from the insect mushroom bodies (e.g. Owald et al.,
487 2015; Saumweber et al., 2018) (Fig. 1D). Indeed, during their learning walks, many ants, not
488 only *Myrmecia croslandi* (Jayatilaka et al., 2018) display regular head and body oscillations,
489 facing alternatively towards and away from the nest (Zeil and Fleischmann, 2019). We
490 assumed in our model that these views form two distinct memory banks: one holding
491 'attractive', nest-directed, views and one holding 'repellent' views pointing away from the
492 nest, and that both sets are used continuously and simultaneously during homing. Our agent
493 compares the current view to both sets of memories at each time step and thus obtains two
494 familiarity values, one for attraction (high familiarity, inhibiting turns) and one for repulsion
495 (high familiarity, triggering large turning amplitudes). Given that both memory pathways have
496 antagonist outcomes, they can be simply integrated by subtracting attractive and repellent
497 familiarity values, resulting in what we called here an 'overall drive' which modulates the
498 amplitude of the oscillator (Fig. 1C).

499 Interestingly, this model closely mimics ant behaviour as documented in our behavioural
500 experiments. If released on a fictive tread-mill (preventing the agent from translating) it
501 displays high amplitude turns when released on top of the nest, and much lower amplitude
502 turns when released further along the homing route. In contrast, when using the 'attractive'
503 memory bank only, the agent produces low amplitude turns at the nest (Fig 5).

504 The behaviour of the agent when combining attractive and repellent views is straightforward
505 to explain (Fig. 1C). At the route release point, facing in the correct direction the simulation
506 generates very small turns because only the attractive memory bank provides a good match.
507 By integrating this with a high unfamiliarity of the repellent memory bank, we obtain a very
508 low overall drive, and thus small turns. However, when released at the nest, whatever the
509 direction the agent faces, there are always both attractive and repellent views that are
510 matching the current view (Fig. 1C). The reason being that these views, when acquired during
511 learning walks, are experienced in multiple compass direction at very closely spaced locations
512 (Fig. 1B). Both attractive and repellent pathways signal high familiarity values and cancel each
513 other out, resulting in large turns.

514

Testing the model's prediction.

Interestingly, the attractive/repellent memory bank model makes a rather counterintuitive prediction, because it relies on the relative difference in familiarities between attractive and repellent pathways and not on the absolute familiarity experienced: the agent's behaviour should be similar when on top of the nest and at a completely unfamiliar location, outside the catchment area of acquired views. At the nest, both attractive and repellent memories result in high familiarity, so their signals cancel each other when integrated (attractive - repellent), resulting in large turns. In completely unfamiliar terrain, both attractive and repellent memories result in very low familiarity, and thus their signals equally cancel each other when integrated (attractive - repellent), resulting also in large turns (Fig. 1C).

As predicted by the model, experiments showed indeed that ants tethered at a completely unfamiliar location exhibit a very similar behaviour to when released on top of the nest: that is, they displayed regular high amplitude path oscillations (Fig. 6).

Integration with path integration.

We did not incorporate integration of path integration information and landmark panorama guidance in our model and so do not at this stage tackle the fact that full vector ants (i.e., those captured with a remaining path integration home vector) showed a small bias towards the home vector direction at the nest location (Fig. 2 & 3, FV vs ZV ants). In *M. croslandi* foragers, as in other ants, path integration information and scene information are integrated (Collett et al., 2001; Collett, 2012; Reid et al., 2013 ; Legge et al., 2012; Narendra et al., 2013; Wystrach et al., 2015; Wehner et al., 2016) with familiar views more strongly weighted – to the degree that a current view providing information on heading direction can completely override conflicting information from path integration (Kohler and Wehner, 2005; Narendra et al., 2013; Zeil et al., 2014). In ants that rely heavily on path integration, this information is more strongly weighted as the length of the vector increases (Wystrach et al., 2015; Wystrach et al., 2019). The bias towards the home vector direction observed here in FV ants fits this current view, which is summarised in a recent model (Hoinville and Wehner, 2018). Also, experienced ants seem to rely less on path integration than naïve ants, and rather display a

search when on unfamiliar terrain (Schwarz et al., 2017), which may explain why path integration information is never strongly weighted in the long-lived *M. croslandi*.

Outlook

Our results may contribute to the lingering debate about the format of spatial knowledge underlying visual navigation in insects and animals in general (see for instance, Cheeseman et al., 2014a,b and Cheung et al., 2014; Warren, 2019). We showed that ants released on top of the nest displayed large turns. These results were clearly at odds with the current 'procedural' models, stipulating that the high familiarity of views at the nest should inhibit turns. In contrast, the ants' behaviour suggested that they could derive positional knowledge from the current views, given the interpretation that the ants searched because they recognised that they were at the nest. Previous results, such as the apparent ability of insects to make shortcuts also favoured explanations assuming 'positional' rather than 'procedural' knowledge (e.g. Cheeseman et al., 2014a,b; Warren, 2019). However, as often in the insect literature (Cartwright and Collett, 1983; Collett et al., 2007; Cruse and Wehner, 2010; Wystrach and Graham, 2012; Narendra et al., 2013; Cheung et al., 2014), an alternative, more parsimonious explanation can also explain our results: ants may simply combine attractive and repellent memories. Importantly, this procedural explanation did not come from actively seeking for it, but emerged from other observations, such as the way in which ants behave when learning views around the nest (Jayatilaka et al., 2018), avoid adverse situations (Wystrach et al., 2019), steer while walking backwards (Schwarz et al., 2017; Schwarz et al., under review) as well as how appetitive and aversive memory pathways are combined in other insects such as flies (Felsenberg et al., 2018) and fly larvae (Eichler et al., 2017).

Our simulation made the unexpected prediction that behaviour in completely unfamiliar terrain should be the same as at the very familiar nest, which we confirmed by subsequent experimentation. Purely scene familiarity-based modelling replicates these results with astonishing detail, providing support for the suggestion that ants during their learning walks, acquire both attractive, nest-directed views and repellent views when pointing away from the nest during systematic scanning movements (Jayatilaka et al., 2018; Zeil and Fleischmann,

2019). It is not clear at present, however, whether all views are memorized irrespective of gaze direction or only when the ants' head is aligned parallel to the home vector (see discussion in Jayatilaka et al., 2018). We show here, at least, that the distinctly different behaviour of ants over the nest location can be replicated if an agent has an attractive and a repellent scene memory bank.

The most parsimonious explanation for our observations is therefore that the ants operate on 'procedural' rather than 'location' information (*sensu* Collett et al., 2002; Wehner et al., 2006; Graham and Philippides, 2017): at both familiar and unfamiliar locations away from the nest they may know where to go, but they do not know where they are. Moreover, the main assumptions of our simulation - attractive and repellent view comparison driving an oscillator - can be tested by a detailed comparison of the gaze and path directions of individually identified ants during their learning walks and during their subsequent approach to the nest, when returning from foraging excursions. Such an analysis may also reveal how ants eventually pinpoint the nest entrance, which none of the current homing models can properly explain.

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Competing interests

No competing interests declared.

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Data availability

Upon acceptance, data will be made available through FigShare. Requests for further information and for original video footage should be directed to and will be fulfilled by the corresponding author, Trevor Murray. Requests for the python code for the model should be directed to Antoine Wystrach.

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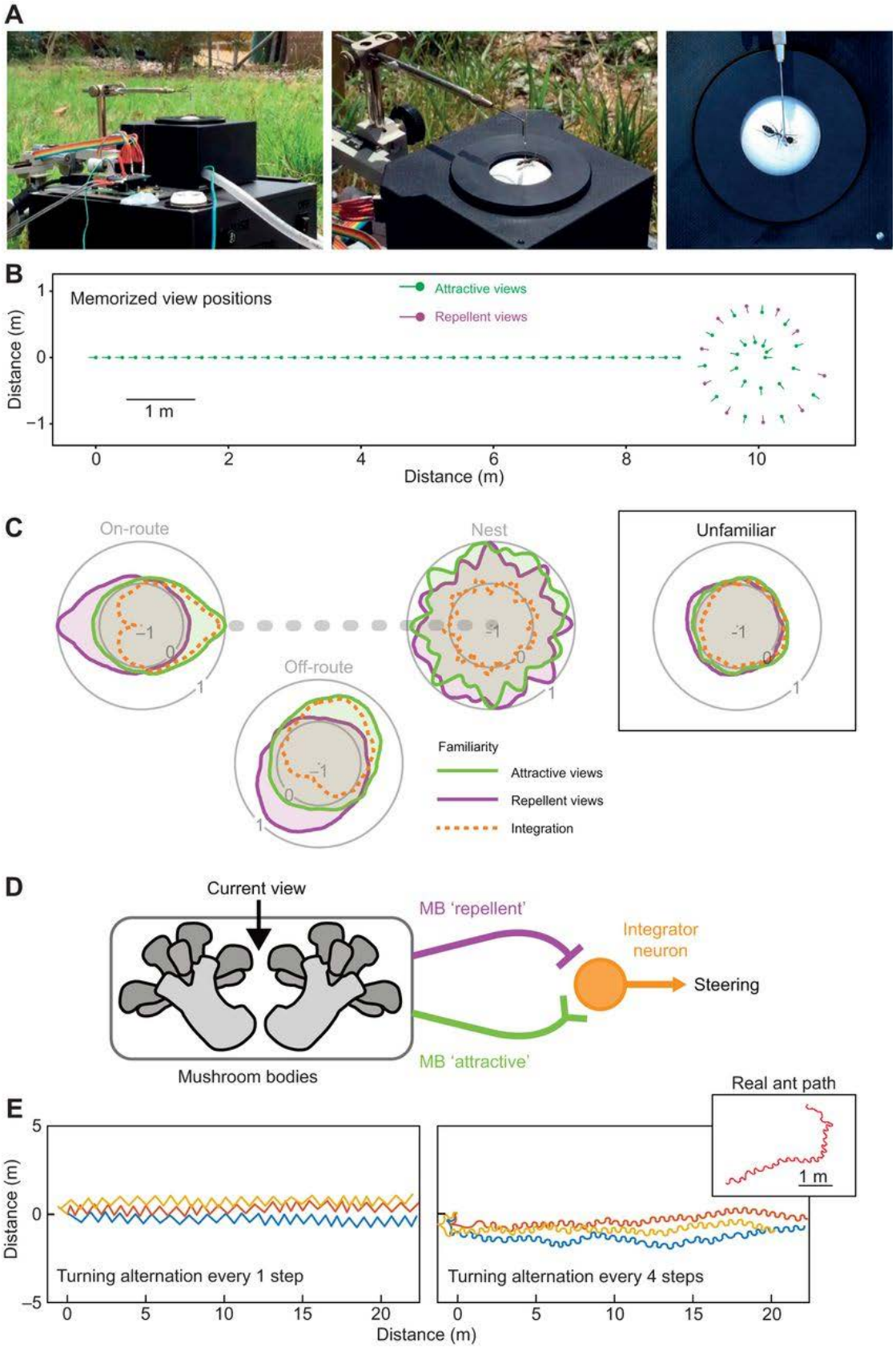
769

Figures and Legends

Figure 1



773 **Figure 1 Experimental set-up and agent-based modelling.** (A) Three views of the air-
774 cushioned trackball contraption and the tethered ant. (B) Schematic map of the attractive
775 (attractive) and repellent (repellent) memorized views along the foraging route and around
776 the nest that constituted the attractive and repellent memory bank. (C) Schematic
777 distribution of familiarity (1) and un-familiarity values (-1) for attractive and repellent views
778 at the four release locations and the result of their integration. Note that distributions at the
779 nest and at the completely unfamiliar site are uniform for different reasons: high familiarities
780 for both attractive and repellent views at the nest and low familiarities for both view sets at
781 the completely unfamiliar site. (D) A 'neuro-schematic' summary of the model comparing a
782 current view with a repellent and a attractive view memory bank and the integration of the
783 output providing a steering command. (E) The paths generated by the simulation reproduce
784 the details of real ant paths better when the regular alternation of path direction is
785 implemented at every 4th step, rather than at each successive step (as has been done in the
786 present study).

787

Figure 2

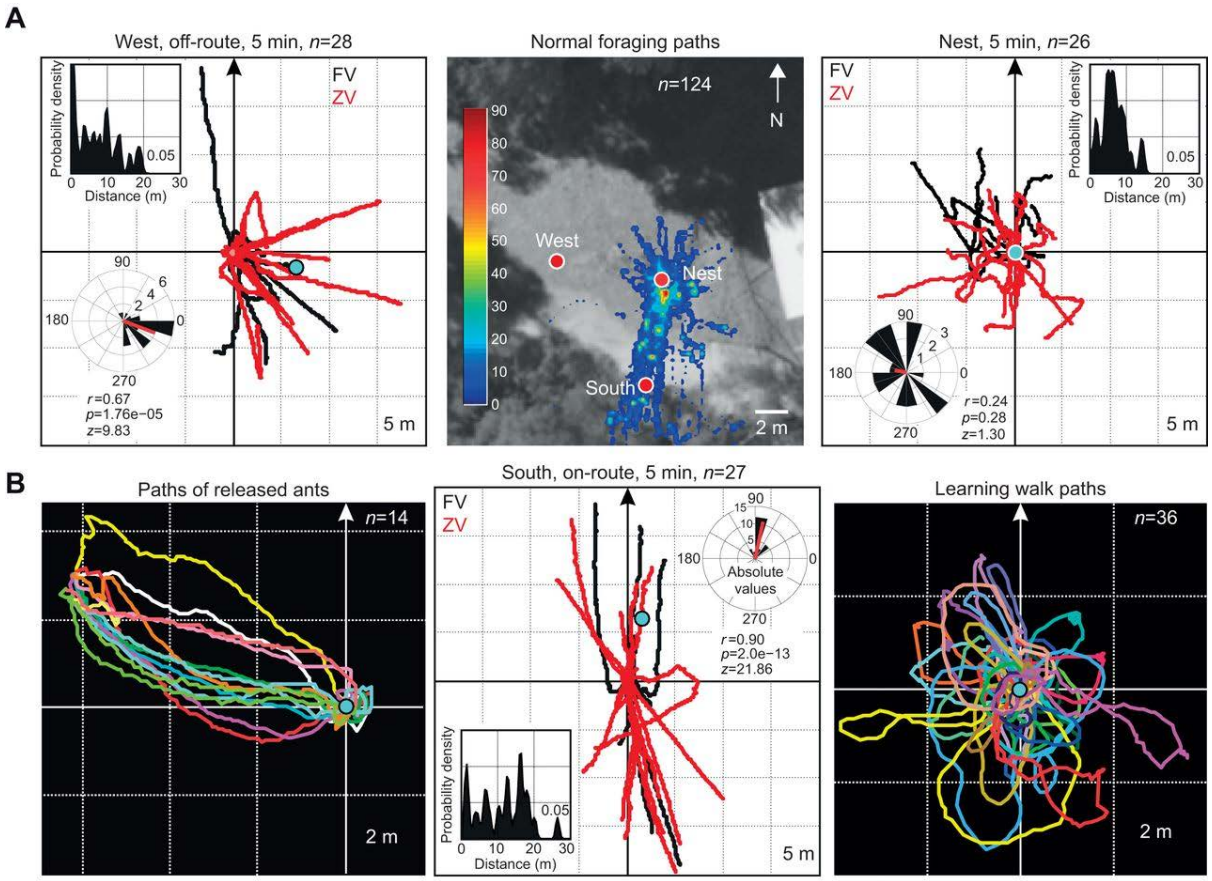


Figure 2 The behaviour of tethered ants at three locations in their natural foraging environment. Top centre panel: Aerial photograph of the nest area with Off-route, Nest and On-route locations marked by red circles. False colour-coded area shows the 2D probability density of 124 outward going paths of foraging ants that operated from this nest and were tracked with Differential GPS over a period of two years. See colour bar for scale and Fig. S1 for individual paths. Intended paths of tethered ants are shown for the Off-route location (top left panel), the Nest location (top right panel) and the On-route location (bottom centre panel), with the paths of zero-vector (ZV) ants shown in red and those of full-vector ants (FV) in black. The nest location is indicated by a blue circle. Insets show for both FV and ZV ants the probability density of virtual distances from the starting point reached after 5 minutes and circular histograms of final bearings with red line indicating the length and direction of the mean vector; r: resulting vector length; p: probability of rejecting hypothesis of uniform distribution and z: z-statistic of Rayleigh test of uniformity. Bottom left panel: Paths of 14 ants released just north of the Off-route location and tracked with Differential GPS. Bottom right panel: Learning walk paths of ants around the nest, recorded with Differential GPS.

805 **Figure 3**

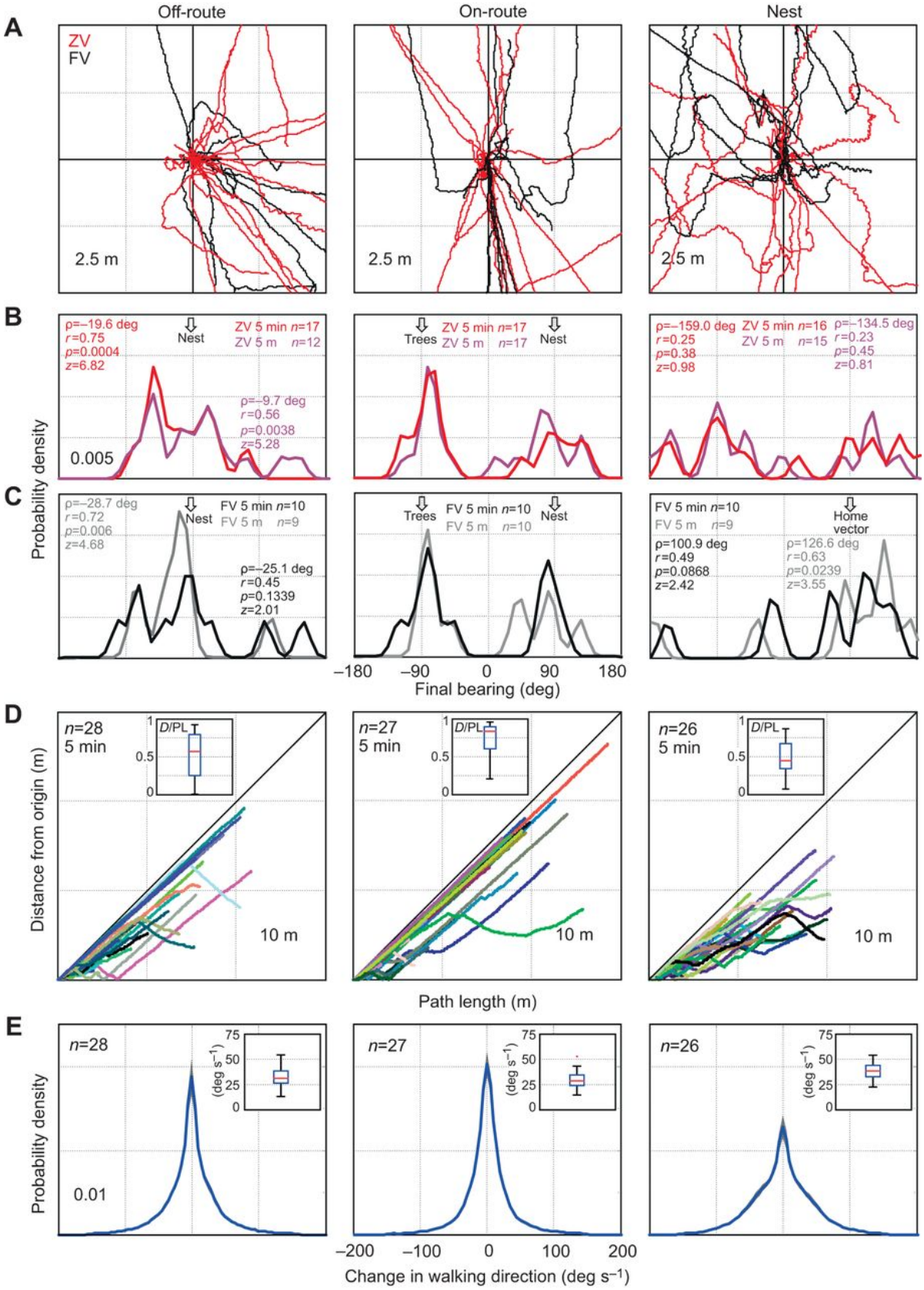


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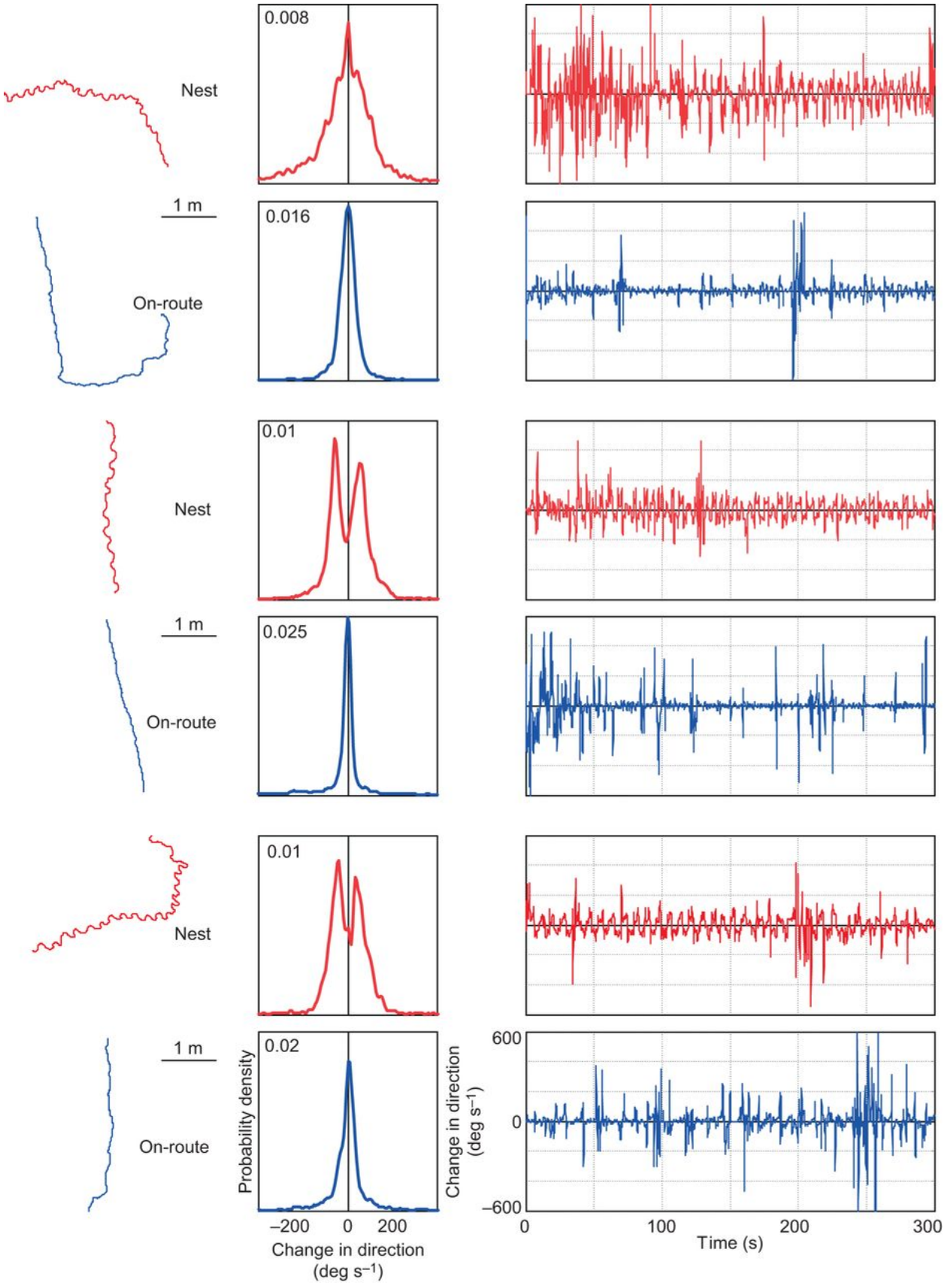
Figure 3 Quantitative analysis of behavioural differences at the Off-route, On-route and Nest location. (A) Initial intended paths of tethered ants at a finer scale. The paths of ZV ants are shown in red and those of FV ants in black. (B) Distributions of final bearings of ZV ants after 5 minutes (red) or when having reached a virtual distance of 5 m from the start (purple) at the three locations. Probability densities determined with 9° bandwidth of the kernel smoothing window; North at +90°. Inset numbers show results of circular statistics (Rayleigh test of uniformity) with rho: mean vector direction; r: mean vector length; p: probability of uniformity; z: z-statistic. Arrows mark the direction of nest and trees. (C) Distributions of final bearings for FV ants after 5 minutes (black) or when having reached a virtual distance of 5 m from the start (grey). Arrows mark the direction of nest, trees and home vector. Otherwise conventions as in (B). (D) Distance from start over path length for the first 5 minutes of paths at the three locations. Paths are randomly coloured. Insets show boxplots with median marked red for the ratios of distance over path length at the end of 5 minutes. See Fig. S2B for statistics. (E) Distributions of changes in walking direction for all 5 min paths at the three locations. Shown are the means of individual distributions (blue) and standard errors in grey (not visible). Insets show boxplots for the distributions of individual means. See Fig. S2C and D for statistics.

825 **Figure 4**



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827 **Figure 4 Ants walk differently at the Nest (red) and the On-route (blue) location.**
828 Shown are path segments on the left, the distributions of changes in walking direction during
829 the first 5 minutes in the middle row and the time series of changes in walking direction over
830 5 minutes on the right for three ants (top, centre, bottom), each recorded at the nest and at
831 the on-route location. Changes in walking direction were determined at 11fps to reduce
832 measurement noise. See Fig. S3 for auto-correlation functions.

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835 **Figure 5**

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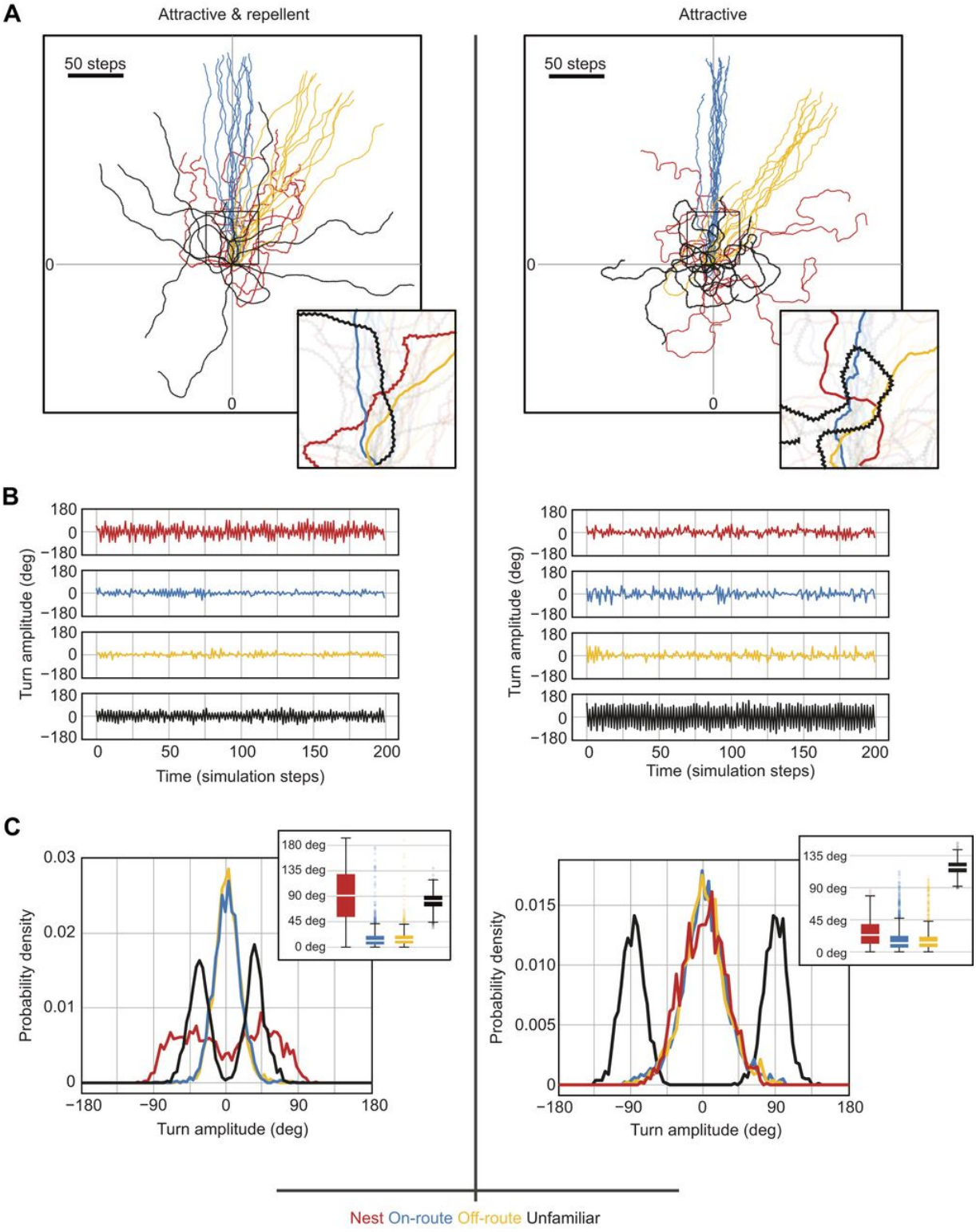

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Figure 5 The results of agent-based visual navigation using both attractive and repellent views (left column) and attractive views only (right column). We simulated ten agents walking 200 steps at each nest (red), on-route (blue), off-route (yellow) and unfamiliar (black) release locations. (A) Resulting paths. Insets show close-up details of example paths. (B) Turn amplitudes over time (simulation steps) for one example at each of the release locations. (C) Probability densities of turn amplitudes at the four release locations. Inset show box and whisker plots for the same distributions.

847 **Figure 6**

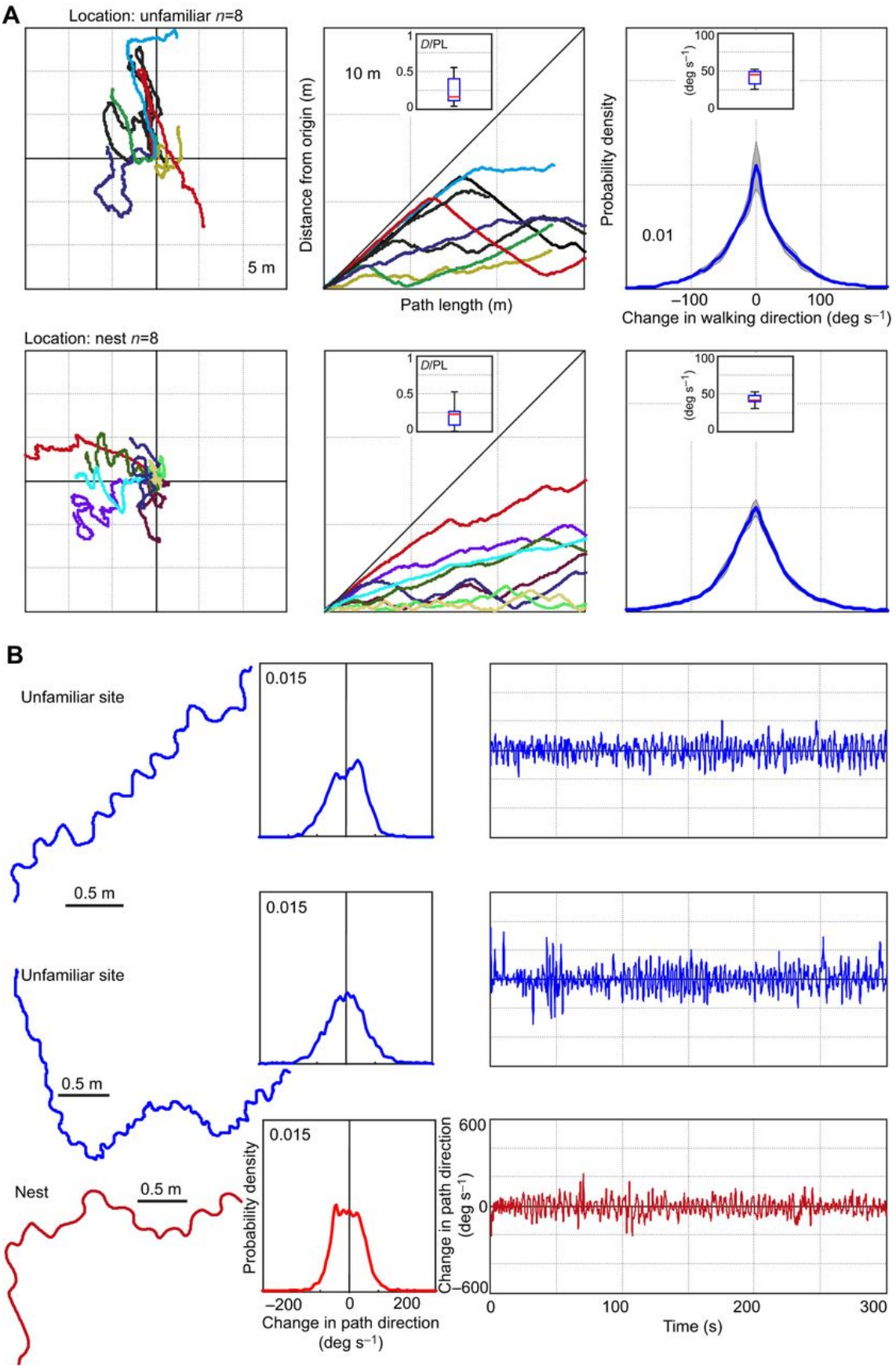

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Figure 6 Ants behave in a similar way at a completely unfamiliar location and at the nest. (A) Top row: Paths (left), distance from start over path length (middle) and probability density of changes in walking direction (right) for 8 tethered ants at a completely unfamiliar location. Bottom row: Same for 8 ants at the nest location. Insets in middle panels show boxplots of final distance to path length ratios after 5 minutes, which are not different between the unfamiliar and the nest location (Wilcoxon Rank Sum test unfamiliar *vs* nest location: p=0.7984, ranksum=71). Insets in right panels show the boxplots of mean absolute values of changes in path direction over 5 minutes, which are not different between the unfamiliar and the nest location (Wilcoxon Rank Sum test unfamiliar vs nest location: p=0.9591, ranksum=67). (B) Example paths (left), probability density of changes in path direction (middle) and time series of changes in path direction (right) for two ants at the unfamiliar site (blue) and one ant over the nest (red).

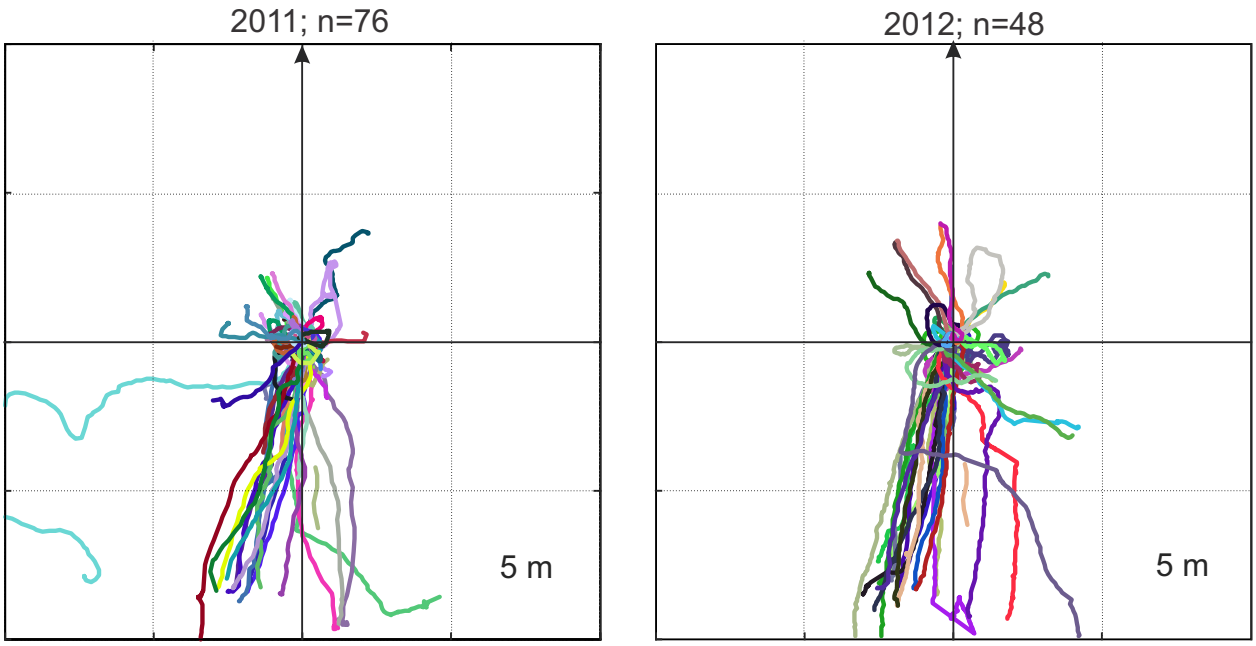

Figure S1 Foraging patterns at the *M. croslandi* nest used in this study. Panels show the individual paths of foragers as they have been recorded with differential GPS over a period of two years. These paths provided the original data for the 2D histogram shown in the middle panel of Fig. 1B.

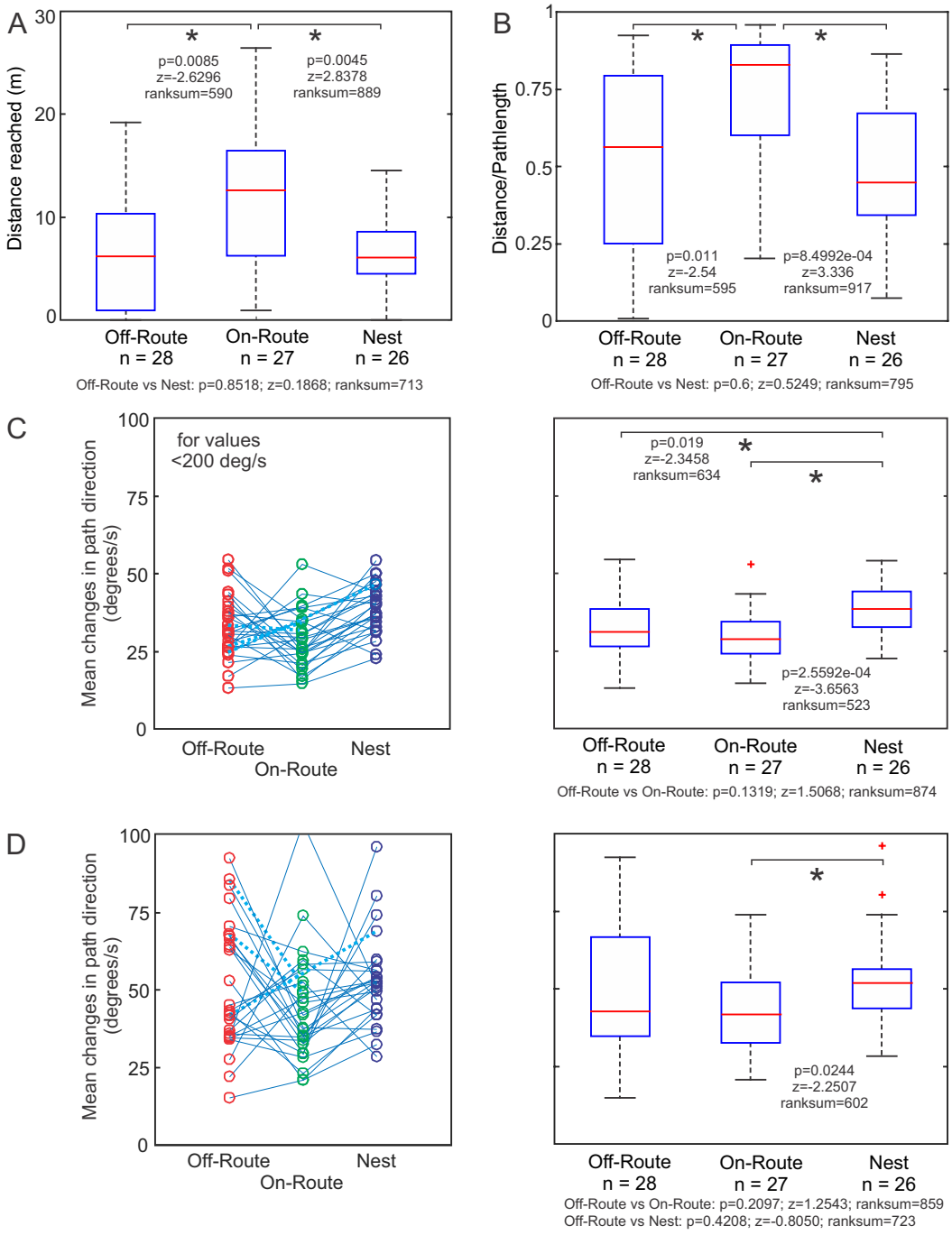

Figure S2 Quantitative analysis of behavioural differences between Off-route, On-route and Nest locations. (A) Box plots of distances reached after 5 minutes at the three locations. Significant comparisons with a Wilcoxon Rank sum test are marked by a star and values are shown inside the panel. Values for on-significant comparisons are shown below the panel. (B) Box plots of distance over path length ratios after 5 minutes at the three locations. Otherwise conventions as in (A). (C) Left panel: Individual means of the changes in path direction (absolute values < 200°/s, determined at 11fps) for the first 5 minutes with means of individual ants connected by blue lines. Dashed lines mark cases where an ant was released at two locations only. Right panel: Boxplots of mean changes in path direction with significant differences as determined by Wilcoxon Rank Sum test marked by a star and values shown as inset. Values for non-significant comparisons are shown below the panel. (D) Same for the means and distributions of all absolute values of changes in path direction. Otherwise conventions as before.

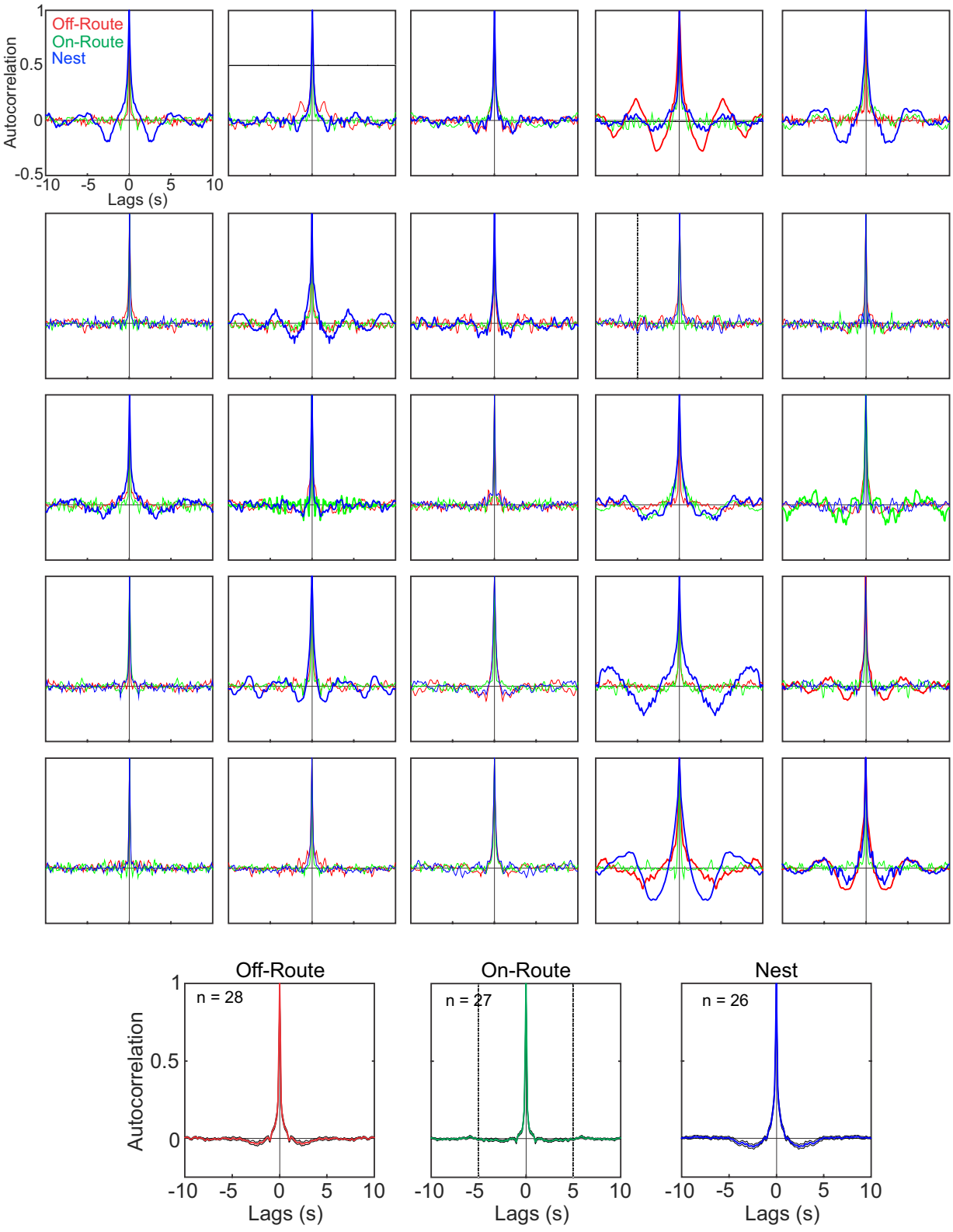

Figure S3 Comparison of auto-correlation functions of 5 minute time series of changes in path direction at the three locations. Off-route: red; On-route: green; Nest: blue for each of 25 ants that were tested at all three locations. Bottom panels show mean auto-correlations for all ants at the three locations.