Path integration in desert ants, *Cataglyphis*: how to make a homing ant run away from home

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Path integration is an ant's lifeline on any of its foraging journeys. It results in a homebound global vector that continually informs the animal about its position relative to its starting point. Here, we use a particular (repeated training and displacement) paradigm, in which homebound ants are made to follow a familiar landmark route repeatedly from the feeder to the nest, even after they have arrived at the nest. The results show that during the repeated landmark-guided home runs the ant's path integrator runs continually, so that the current state of the homebound vector increasingly exceeds the reference state. The dramatic result is that the homing ants run away from home. This finding implies that the ants do not rely on cartographic information about the locations of nest and feeder (e.g. that the nest is always south of the feeder), but just behave according to what the state of their egocentric path integrator tells them.

Keywords: desert ant; *Cataglyphis*; orientation; path integration; vector navigation; landmark navigation

1. INTRODUCTION

In desert ants, *Cataglyphis*, path integration (vector navigation) is the predominant mode of navigation and the only one available to the ants in landmark-free terrain such as the North African salt-pan areas inhabited by *C. fortis*. The path-integration process results in a global vector that continuously connects the ant with its starting point and—reversed in sign—later leads the ant to its last visited feeding site (for recent reviews of path integration in insects see Wehner (1992), Collett & Collett (2000) and Wehner & Srinivasan (2003)). In cluttered environments, as in the low-shrub semi-desert surrounding the salt-pans, *Cataglyphis* additionally relies on landmark-based route memories (Wehner *et al.* 1996). In this type of environment the ants usually follow multi-segment paths by memorizing a series of landmark views, each associated with a local vector (for local vectors in *Cataglyphis* see Collett *et al.* (1998, 2001), Andel & Wehner (2001) and Bisch-Knaden & Wehner (2001)). The ant can later follow these landmark-based routes even if its path integrator has previously been reset to the zero state, i.e. even if the ant has completed its homeward run and is captured just before vanishing into the nest hole. Displaced back to the feeder and released there, it negotiates its habitual route with the same precision as it does when its home vector is still fully available at the starting point (Wehner *et al.* 1996; Kohler & Wehner 2004). We now ask whether the vector-navigation system keeps running while the ant is following its habitual homebound route after its path integrator has been reset to the zero state, i.e. when, prior to displacement, the current state of the vector has already matched the stored one, and when the ant is navigating exclusively by landmark guidance. If the path integrator keeps running, the global vector of the homebound ant should now point in the opposite direction, that is, away from home.

We trained individual ants within a narrow linear channel provided with a series of conspicuous landmarks to run back and forth between the nest and an artificial feeder. When later transferred from the feeder to a test channel aligned with the training channel but devoid of landmarks, these (+1) vector ants follow their home vector. We refer to these ants as (+1) vector ants, because upon release, the output of their integrator, that is, the difference, D, between the reference vector ($R = +1$ corresponding to the full vector pointing towards home from the current feeding site) and the state of their current vector ($C = 0$, as the ants have not yet started their home run), is $D = R - C = +1$. If in the training channel the ant has returned to the nest ($R = 1$, $C = 1$, hence $D = 0$) and is displaced from there back to the feeder, it will return home again, this time by referring exclusively to its landmark-based route memory. While the ant performs this landmark-guided home run, its path integrator could either be shut off (hypothesis I) or be running uninterruptedly (hypothesis II). In the latter case, the path integrator of the ant when it arrives at the nest for the second time should indicate $D = -1$ ($R = +1$, $C = +2$, because the ant has run the feeder-to-nest distance twice). This outcome would create the somewhat paradoxical situation that the food-laden and hence homeward-bound ant, when transferred to the landmark-free test channel, should run away from home. This is indeed what occurs.

2. MATERIAL AND METHODS

(a) *Species and experimental design*

Ants, *C. bicolor*, were trained to visit a feeder placed at the end of a 4 m long, 7 cm wide and 7 cm high aluminium channel (figure 1). The nest was located in a polythene box connected by a tube to the other end of the channel. Pairs of landmarks were placed in the channel every 0.5 m. These signposts were made as conspicuous as possible in the feeder-to-nest direction and as invisible as possible in the nest-to-feeder direction, so as to produce a directionally defined landmark route.

Zero vector ants were produced by capturing trained ants after they had arrived in the nest-box. By displacing them back to the
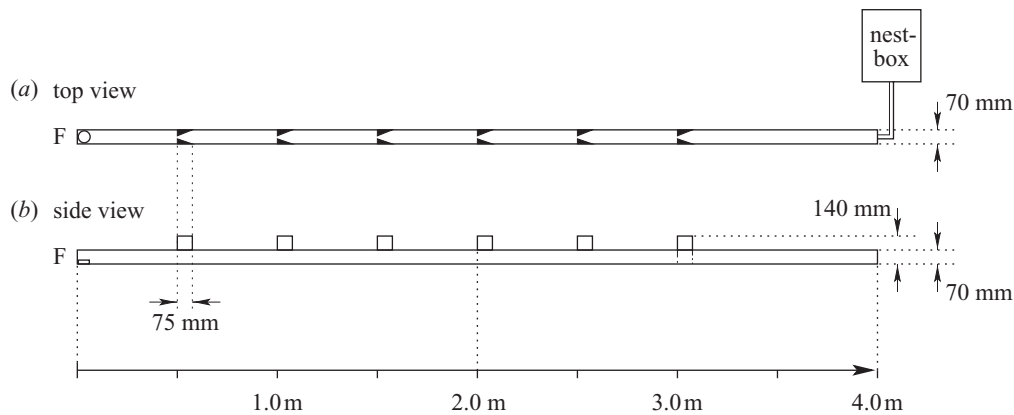
Figure 1. Experimental set-up of the channel experiments. On their homebound runs from the feeder (F) to the nest the ants had to run through 20 mm wide slit-like openings between pairs of conspicuous landmarks (columns; 25 mm wide, 140 mm high), which were painted black on the side facing the feeder. (*a*) Top view, and (*b*) side view.
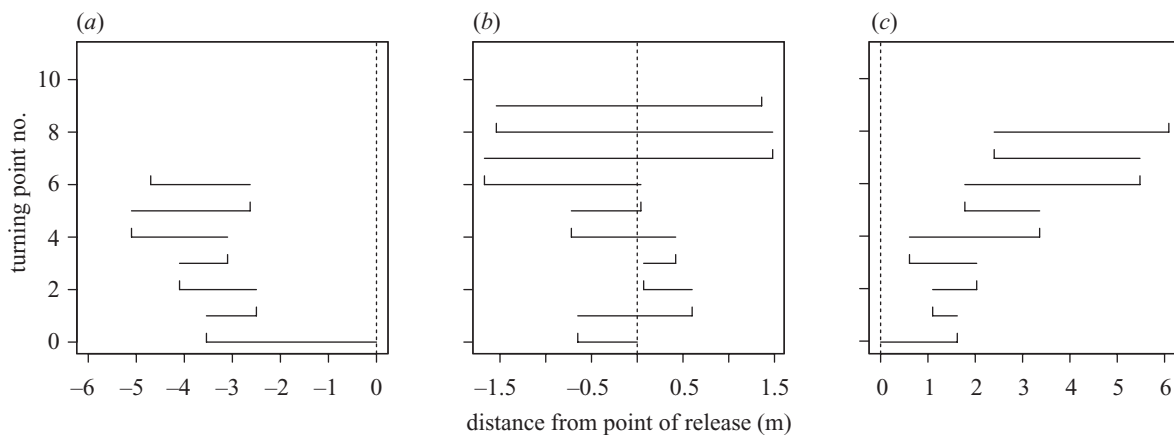

Figure 2. Three individual test runs with the turning points exhibited by the ants during the 3 min test periods. Point of release is at '0 m'; nest direction is towards the right. (*a*) Series −1, (*b*) control series 0 and (*c*) series +1.

feeding site and capturing them in the nest-box for a second time, (−1) vector ants were obtained (for rationale see § 1). Repeating this procedure four and seven times in a row yielded (−3) vector and (−6) vector ants, respectively. During the training phase in the landmark channels, the feeder-to-nest running times did not differ among series +1, 0 and −1 (median = 1.02 min, first and third quartiles = 0.84–1.44 min, $n = 165$, $p = 0.82$; Kruskal–Wallis test), or among series −2, −3, −4 and −5 (median = 1.46 min, first and third quartiles = 1.11–1.95 min, $n = 78$, $p = 0.08$; the difference between this and the former group is significant at $p < 0.001$; Mann–Whitney U-test). Intentionally, we let the ants enter the nest after each displacement run, i.e. captured them only after they had passed the entrance tube of the nest. This experimental paradigm allowed us to check whether the arrival at the nest would reset, or shut off, the ant's path integrator. In an additional experiment, however, the ants, when displaced back within the training channel, were allowed to run in the home direction for only 2 m rather than the full 4 m distance to the nest, and had to do so eight times. Hence, their landmark-guided runs covered the same total distance (12 m) as those of the (−3) vector ants mentioned above, but during the training-and-displacement procedure they never came close to the nest entrance. These ants are referred to as (−3r) vector ants. In all experiments only those ants that had continually carried their food item were finally included in the analyses.

(**b**) *Test procedure and statistical analysis*

Immediately following the training-and-displacement paradigm described above the ants were tested in a 12 m long channel devoid of landmarks and orientated parallel to the training channel. Upon release, they started to run back and forth within this test channel and in doing so exhibited clear directional preferences. The point of release within the test channel was varied among and sometimes even within the different test series. Some of the (−6) and (−3r) vector ants reached the end of the test channel, so that in these series the recorded distances that the ants had run in the off-home direction were even smaller than the distances the ants would have run under completely unconstrained conditions. The ants' turning points within the test channel were recorded over periods of 3 min. Based on these recordings for each ant, the turning point with the maximal distance from the point of release and the mean of all turning points were determined. These data (one data point per ant) were not normally distributed (Shapiro–Wilk normality test). The different test series were compared using the Mann–Whitney and Kolmogorov–Smirnov tests. Unless otherwise stated, statistical significances apply to both the maximal distances and the means of all turning points.

3. RESULTS

If ants have returned from the feeding station and arrived at the nesting site and are displaced directly into

the landmark-free test channel, they search almost sym-metrically about the point of release (figure 2b). In these ants the current state matches the reference state of the home vector (hence 'zero vector ants', for conventions see § 1). This is in accord with the observation that the dis-placed ants search systematically about the point of release. However, if upon arrival at the nest the ants are displaced back to the feeder, from where they perform a second home run (this time guided exclusively by the row of landmarks), and they are then captured at the nesting site a second time and displaced to the test channel, they select a 'homing direction' that leads them away from home (see figure 2a,b and series −1 in figure 3b; signifi-cantly different from zero vector ants according to the Mann–Whitney U-test: $p < 0.005$, $n = 31$). Repeating this displacement paradigm more than once, e.g. letting the ant home exclusively by landmarks three or six times, leads to even longer runs in the direction opposite to home (series −3 and −6 in figure 4; all series significantly differ-ent from each other according to the Mann–Whitney U-test: $p < 0.01$, $n(−3) = 31$, $n(−6) = 12$). By contrast, and as expected, the ants that were displaced to the test chan-nel immediately after their first arrival at the feeder selec-ted the homeward direction (series +1 in figure 4; significantly different from zero vector ants according to the Mann–Whitney U-test: $p < 0.001$, $n = 30$).

Although the ants tested in the (−1), (−3) and (−6) paradigms run in a direction leading away from home, they do not run in this direction as far as one would pre-dict from the total distance they have covered during their repeated landmark-guided runs in the training channel. In series −1, −3 and −6 these distances are 4 m, 12 m and 24 m, respectively. Compared with the (+1) control ani-mals the test ants reached 70%, 35% and 31% of the maximal distances and 54%, 37% and 41% of the mean distances, respectively. One reason for the observed shorter running distances could be the fact that prior to their displacement back to the feeder the ants had arrived at the nesting site, and this experience could somehow have influenced the path-integration system. Hence, in series −3r we prevented the ants from arriving at the nest during their landmark-guided home runs by letting them return in the homeward direction for only half of the feeder-to-nest distance before capturing them and displa-cing them back within the channel but displacing them twice as many times as in series −3. When later tested in the landmark-free channel the ants ran away from the nest for significantly larger distances than they did in series −3 (see figure 4; Mann–Whitney U-test $p < 0.01$), but still did not reach the 12 m mark (Mann–Whitney U-test $p < 0.001$, $n = 14$). The main reason for the observed undershooting associated with the conspicuous back-and-forth locomotor movements in the test situation might be the absence of the landmarks in the test channel. This back-and-forth running behaviour leads to the broad spatial distributions of the ants' turning points shown in figure 3 (see also § 4).

In contrast to all other test series, in series −3r many ants exhibited a striking behavioural trait, not to our knowledge hitherto observed in any *Cataglyphis* species: having repeatedly been forced to navigate home by land-mark guidance in a direction opposite to the one indicated by its increasingly larger path-integration vector and not
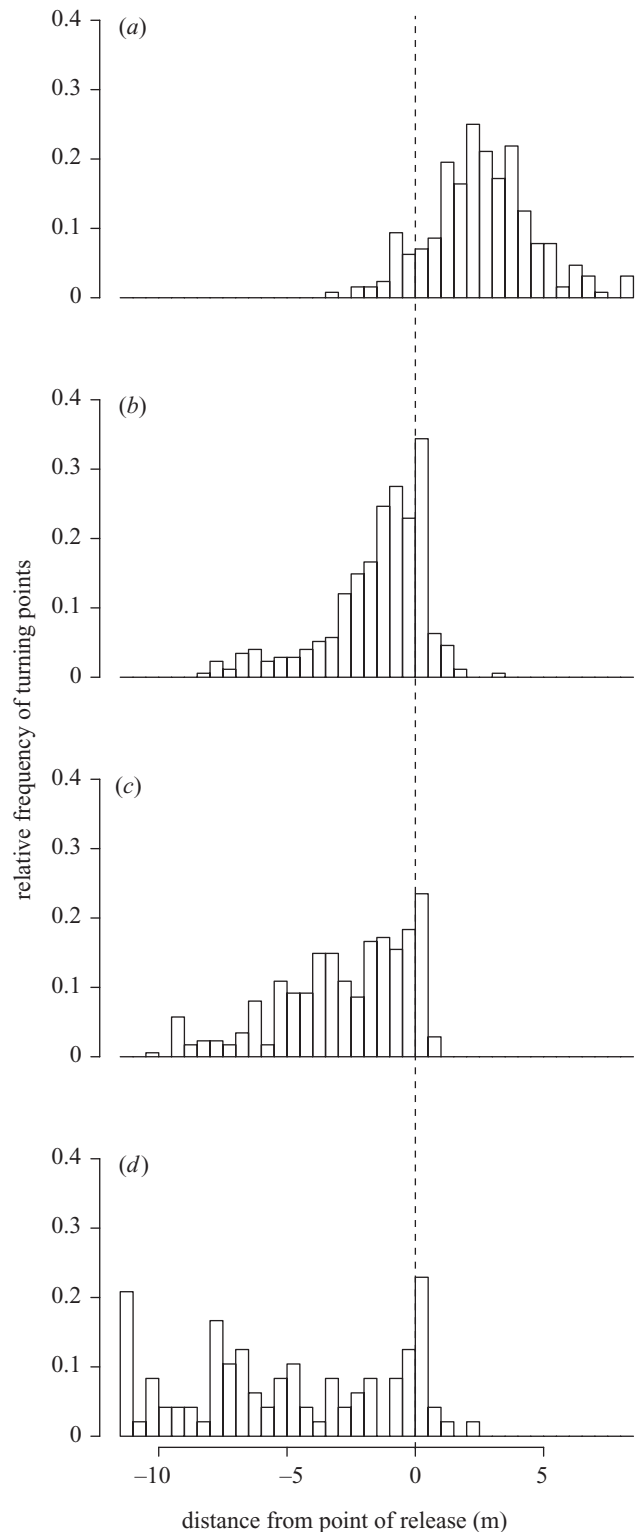
Figure 3. Histograms of all turning points exhibited by the ants during the 3 min test periods. Nest direction is towards the right. Bin size = 0.5 m. (a) Series +1, (b) series −1, (c) series −3 and (d) series −6. The leftmost bin in the −6 condition shows the ants reaching the end of the test channel.

reaching the nest, these ants suddenly stopped walking, dropped their food item, stood still and hardly displayed any escape behaviour when approached by the experimenter. It goes without saying that these ants could not be tested further.
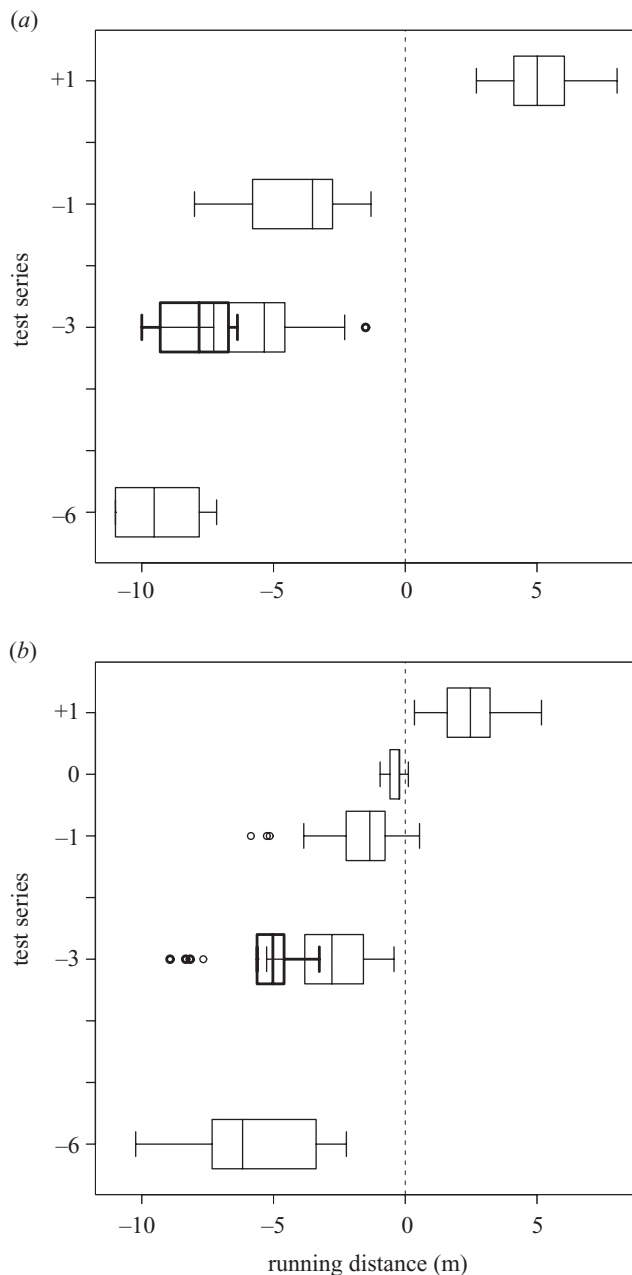
(a)

(b)

Figure 4. Box plots of the ants' turning points (U-turns) within the test channel: (a) maximal outer turning points and (b) means of all turning points exhibited by the ants within 3 min test periods. Positive running distances (abscissae) mean runs in the direction towards the nest; negative running distances mean runs in the direction away from the nest, i.e. towards the feeder. The test series labelled +1, 0, −1, −3 and −6 (see ordinate) refer to the tests performed with the (+1), zero, (−1), (−3) and (−6) vector ants, respectively. The bold box plot represents the data obtained from the (−3r) vector ants. The box plot representations include the medians, the 25% and 75% percentiles, whiskers extending to the most extreme data points that are no more than 1.5 times the interquartile range from the median, and the outliers.

4. DISCUSSION

The experimental paradigm applied here allowed us to test, in a rather straightforward way, whether the ant's path-integration system keeps running, even after the ant has arrived at its goal, i.e. after the current state of the

home vector had matched the stored reference state, and is then forced, by experimental interference, to run home again for a second (or nth) time by referring to landmark cues alone. The results clearly show that this is the case. If a homing ant is subjected to this experimental paradigm, the output of its path integrator can even guide the food-laden animal away from home. This result implies that the path integrator is continually running and not shut off after the ant has reached its nest-box.

In preliminary experiments performed at our North African field site we had already shown that the ant's path integrator is continuously updated even if, owing to a particular test procedure, the homing ants follow a learned landmark route that deviates from the direction indicated by the path integrator (Sassi & Wehner 1997). These and other more extended series of experiments (Collett *et al.* 2003) show that ants do not recall the (long-term) reference state of their path integrator when at a particular site, but always display the home vector that is based on the immediately preceding outward journey. Under all conditions tested so far the ant's global home vector reflects the difference between the reference state and the continually updated current state of the path integrator (Collett *et al.* 1999). As shown here, this mode of operation may even lead to the paradoxical situation in which a homing ant runs in the direction opposite to home.

However, even though in our experimental paradigm the homing ants were made to run away from home, they did not do so for the complete distance given by the difference between R and C. This can be predicted from the experimental paradigm adopted for generating (−1), (−3) and (−6) vector ants: the ants had to be trained in a channel provided with conspicuous landmarks (pairs of columns) and were later tested within a channel completely devoid of such landmarks. We know from open-field experiments in the ants' natural environment (Wehner 1982; I. Flatt and R. Wehner, unpublished observations) that, under these conditions, when the ants are trained in landmark-rich surroundings and subsequently tested in landmark-free areas, they do not run off their home vector completely, but start to run back and forth in the general direction of the home vector long before they have arrived at the fictive position of the nest. In the present experiments this can be observed even in the (+1) vector ants (figure 2c). In contrast to ants that had been trained and tested in channels that were devoid of particular landmarks in both the training and the test situations (Sommer & Wehner 2004), the (+1) vector ants did not complete their 4 m vector before starting their oscillatory locomotor movements. This effect becomes more pronounced the more often the ants have been trained to follow their home route by relying on landmark guidance alone (figure 3).

Finally, the most general conclusion that can be drawn from the ants' misguided behaviour as elicited by our experimental paradigm is that the animals do not acquire and use information about the absolute positions of nesting and feeding sites (say, information about the nest being located south of the feeder), but just information about the landmark route leading from the feeder to the nest, and, if such landmarks are removed, information is provided exclusively by the current state of the path integrator. This conclusion is in agreement with the view that

ants rely on procedural information—what to do next when experiencing a particular visual (external) cue or a particular (internal) state of the path integrator—rather than on positional information encoded within an allocentric system of reference (Collett *et al.* 2003; Wehner 2003).

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