Vector-based and landmark-guided navigation in desert ants of the same species inhabiting landmark-free and landmark-rich environments

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Abstract

The central Australian desert ant *Melophorus bagoti* lives in a visually cluttered semi-arid habitat dotted with grass tussocks, bushes and trees. Previously, Bühlmann et al. (Bühlmann et al., 2011) have shown that this species has a higher propensity to switch from vector-based navigation to landmark-guided navigation, compared with the North African desert ant *Cataglyphis fortis*, which usually inhabits a visually bare habitat.

Here we ask whether different colonies of *M. bagoti*, inhabiting more and less cluttered habitats, also show a similar difference. We compared ants from typically cluttered habitats with ants from an exceptional nest located on a open field largely devoid of vegetation. Ants from both kinds of nests were trained to forage from a feeder and were then displaced to a distant test site on the open field. Under these conditions, ants from cluttered habitats switched more readily from vector-based navigation to landmark-guided navigation than ants from the open field. Thus, intraspecific differences due to the experience of particular landmarks encountered *en route*, or of particular habitats, ride on top of previously found interspecific, inherited differences due to the evolutionary history of living in particular habitats (Bühlmann et al., 2011).

Key words: ant, vector-based navigation, path integration, landmark guidance, intraspecific comparison

Running head: Vector- and landmark-based navigation
Introduction

One of the big open questions in research on ant navigation in particular, and insect navigation in general, is the relative role that path integration, also known as vector-based navigation, and landmark guidance routines play in the animal’s overall behaviour. As previous studies have shown, landmark-defined route guidance (LG) usually outcompetes guidance based on the path integration home vector (HV) (Andel and Wehner, 2004; Kohler and Wehner, 2005; Wystrach et al., 2011), but even if the ant’s behaviour is governed by LG, the path integrator continues to run in the background until the ant has finally returned to and re-entered the nest (Sassi and Wehner, 1997; Andel and Wehner, 2004; Knaden and Wehner, 2005; Knaden and Wehner, 2006).

Results such as the ones obtained in the LG-HV competition experiments cited above as well as other indications (Wehner et al., 1996; Narendra, 2007a; Narendra, 2007b) raise the question of whether (i) ants of colonies inhabiting cluttered or landmark-free environments differ in the relative weights they attribute to HV and LG routines (intraspecific, environment-dependent differences), and whether (ii) ants of different species or even genera differ in this respect as well (interspecific, inherited differences). The latter has already been shown to be the case. The North African Cataglyphis fortis, which inhabits largely landmark-free salt-pan areas, and the central Australian Melophorus bagoti, which inhabits richly cluttered, steppe-like environments, were subjected to the same experimental paradigm. When trained to run along an artificial landmark corridor and later transferred to a landmark-free test field, C. fortis relied much more strongly on its HV routine than M. bagoti did (Bühlmann et al., 2011). In the present study we test whether similar differences occur also
intraspecifically, as a function of natural variations in the landmarks encountered by the ants. We do so by comparing ants of the species *M. bagoti* taken from colonies located either in an open-field area (‘open-nest ants’) or in very cluttered environments (‘cluttered-nest ants’), and testing them all at the same location on the open field, some distance from the open nest.

Disparate results from past studies suggest that full-vector ants from cluttered nests would not run as far before engaging in search for landmarks they usually encountered *en route*. Thus, Bühlmann et al. (Bühlmann et al., 2011) displaced the open-nest ants (the same nest as used in this study), and found that they ran most of the accumulated vector. In another condition from the same study, artificial landmarks placed along the training corridor were presented to ants of the same nest. When these artificial landmarks were absent on a test on the open field (but distant from the nest), the ants did not run as far before engaging in searching behaviour. This result suggests that experience with artificial landmarks matter.

But artificial landmarks are not natural, and affect the foraging behaviour of the ants in other ways. For example, the paths of travel are straighter when the ants travel down a corridor of landmarks as opposed to travelling on open field (Bühlmann et al., 2011). Would experience with natural landmarks produce a similar effect, leading ants tested on the open field to run a shorter distance, and perhaps a more tortuous path, before engaging in a search, in comparison with ants from the nest on the open field, which experience nothing by way of landmarks along the route? Based on comparisons between studies, we predicted so. Narendra (Narendra, 2007a) displaced ants from nests in typical cluttered habitats, to a different but also cluttered habitat, and found that the ants ran only about 44% of the accumulated vector before engaging in search, a much
shorter proportion than the *M. bagoti* ants in Bühlmann et al.’s (Bühlmann et al., 2011) study. In this account, we set out to test this hypothesis of intra-specific variation in navigation within one study.

For the intraspecific comparison we selected *M. bagoti* as the test species, because even though this species usually inhabits cluttered environments, one nest could be found that was located on an open plain largely free of vegetation (see already Bühlmann et al., 2011). On the other hand, *C. fortis*, the typical open-habitat salt-pan ant, could not be found in the type of cluttered environment characteristic for *M. bagoti*. Furthermore, it should be noted that the interspecific comparison performed by Bühlmann and coworkers was actually an intergeneric one (a *Cataglyphis* versus a *Melophorus* species). It was originally conceived as an interspecific comparison performed within the genus *Cataglyphis*, i.e., between *C. fortis* and the cluttered-habitat species *C. bicolor*, but as recently colonies of the latter species could not be located at proper sites in the vicinity of our field station at Maharès, Tunisia, we used *M. bagoti* rather than *C. bicolor* for the interspecific study to which we repeatedly refer in the present account.

**Methods**

Species

The red honey ant *Melophorus bagoti* Lubbock 1883 is found over a large expanse of semi-arid Central Australia. Like all members of the genera *Cataglyphis* and *Ocymyrmex* in the Afro-Asian and southern African deserts, respectively (Wehner, 1987; Wehner and Wehner, 2011), ants of this *Melophorus* species fill a thermophilic niche (Christian and Morton, 1992), and come out to forage in the heat of the day.
during the summer months. Foragers carry home various plant materials, nectar, and also scavenge for dead arthropods (Muser et al., 2005). The study was conducted in January and February of 2008.

Experimental location and set up

Thanks to permission from the Alice Springs Airport Authority, experimentation took place at and around a field cleared of most vegetation, normally used for launching hot-air balloons. The balloon-launching field was surrounded by fairly uniformly tall trees, but the field itself was devoid of the usual vegetation of the area (Fig. 1a). On the balloon-launching field we could find one active nest, which served as the ‘open’ nest. All around the balloon-launching field, the vegetation was typical for the area, cluttered by tussocks mostly of the invasive buffel grass (*Cenchrus ciliaris*), along with bushes and trees of *Hakea* and *Acacia* genera (Fig. 1b). The area around the balloon-launching field was wild and not maintained at all, and as a result contained even more plants and leaf litter than the sites on well maintained properties used for past research on *M. bagoti* route-learning (Wystrach et al., 2011; Wystrach et al., 2012). Around the balloon-launching field we located 3 nests (‘cluttered’ nests) active enough and suitable for experimentation. Suitable meant having no trees or large bushes 12 m south, because we set up a feeder for each nest 12 m south. This consisted of a small plastic container (~20 cm square) sunk into the ground in which crumbs of cookie were placed.

Insert Fig. 1 about here

On the balloon-launching field, a test grid of 1-m squares was constructed ~70 m from the open nest. The grid consisted of strings wound around tent pegs stuck in the ground, 20 m long north-south, 10 m wide east-west. The path of ants on tests was traced on gridded paper.
Procedure

For logistical reasons, one nest was experimented on at a time. An ant that first arrived at the feeder was painted with a dot of paint and then followed back to the nest. For cluttered nests, which were found in sufficient density, this was crucial for ensuring that the ant came from the experimental nest. A painted ant that returned was painted again with more dots for individual identification, and allowed to run home with a piece of food. On its fifth trip to the feeder, the ant was captured in a tube and carried to the test field in the dark. After the ant had grabbed a piece of cookie, it was released at the southern end of the test grid, in the middle. Its path was traced on gridded paper for 5 min, after which the ant was captured and returned to her nest. Each ant was tested only once (n = 20 for the open nest, n = 10 for each cluttered nest).

For the three cluttered nests, we also obtained their headings from the feeder in the training area, using different ants from the tested ones (n = 10 for Nest1 and Nest3, n = 9 for Nest2, which was becoming inactive). This was done by timing the ant as she ran home from the feeder, and marking where the ant was 15 s into her journey. The direction from the start of the run to this point was taken as an estimate of heading direction.

Data analysis

Each path from the test field recorded on paper was scanned as an electronic file and then digitised using the software GraphClick™, which delivered the x and y coordinates of successive points of the path in metres. We digitised the path into as many straight segments as can be readily resolved on the screen at 100% viewing scale. We stopped digitising when the path went off the grid.
The point at which the ant first ended her straight run and started searching was then determined according to criteria closely matching those used by Bühlmann et al. (Bühlmann et al., 2011). Some ants did some turns and loops right at the beginning of the test, and we ignored those, not counting any turns and loops within the first metre of travel. Otherwise, the start of search was defined as the point at which the ant turned by at least 60° and did not revert back to the original direction of travel for at least 3 m, or if the ant turned and made a loop (crossed her original path).

The beeline distance \( d_0 \) was used as a measure of the distance travelled to the fictive nest before searching. This was the \( y \) coordinate of the start of search, i.e., the projection of the start of search onto the line connecting the start of the run and the fictive nest. The path length \( l \) to the start of search was calculated by adding up the lengths of all the segments delivered by GraphClick™. A straightness measure was defined as the straight-line distance, \( d \), from the start of the run to the start of search divided by the path length, i.e., \( dl / l \). A perfectly straight run has a straightness measure of 1.0.

For a directional measure, we could not use Bühlmann et al.’s (Bühlmann et al., 2011) definition of where the path intersected a circle of 5 m radius because not every path was 5 m long. Instead, we took the direction to be defined by the point closest to 80% of the path length from the start of the run to the start of search. We did not want to use the start of search to define direction as the ant might have started veering off course on a search already when she met the criterion for the start of search. In point of fact, the two definitions of direction produced similar results (Pearson correlations over 0.9). The target direction was defined as 90°, with positive errors counterclockwise, following traditional trigonometric conventions.
Directional measures were compared using circular statistics (Batschelet, 1981). For cluttered nests, the distributions of directions at the training site and on the test field were compared, for each nest singly. The cluttered nests as a group were then compared with the open nest in their performance on the test field. The Watson-Williams test was used to compare differences in mean heading direction, while the K-test was used to compare differences in directional scatter.

Linear measures were compared using the standard parametric techniques of analyses of variance (ANOVA). Ants that did not run onto the test grid, but headed in the direction opposite to the fictive nest at the start of their runs, were not counted. Since only ants from cluttered nests did this, this exclusion biases against finding differences in lengths between open and cluttered nests. We used O’Brien’s (O’Brien, 1979) test, available in the statistical package JMP™ (SAS, 2002), to compare variances between groups. We prefer this test because it is not overly influenced by occasional outliers, compared with other tests such as Levene’s or the $F_{\text{max}}$. Should a significant difference ($p < 0.05$) in variance be found, Welch’s ANOVA, also available in JMP, was used to test for differences in means. Otherwise, a standard ANOVA was used.

**Results**

In general, the ants ran off in the direction towards the fictive nest on a test, sometimes after a few twists and turns at the start (see Fig. 2 for some examples). All 20 ants from the open nest did this. A few ants from cluttered nests, however, did not run onto the test grid, but in the opposite direction, that is, somewhere in the half-circle opposite to the direction to the fictive nest (2, 1, and 2 out of 10 ants in Nest1, Nest2, and Nest3, respectively). Thus, few ants from any nest failed to run onto the test grid.
(Fisher’s exact test between open and cluttered nests, $p = 0.075$). On the whole, the ants were oriented in the general direction of the fictive nest (Fig. 3). Cluttered Nest1 showed a small bias to the left of the nest/fictive nest, both on the training and test fields. At their training site, they had an obstacle (tussock) in the direct path between feeder and nest, which the ants detoured around to the left.

**Insert Figs. 2 and 3 about here**

**Directions**

We first compared mean directions of the cluttered nests, on the training field vs. the test field. These were not significantly different for any nest (Watson-Williams test, $p’s > 0.24$). In directional scatter, all three nests showed significantly more scatter on the test field than on the training field (K-test, Nest1: $p = 0.034$, Nest2 and Nest3: $p < 0.001$). Thus, on the unfamiliar test field, headings were more variable across ants.

We then compared the directions of travel on the test field of the open nest vs. the three cluttered nests combined. Because Nest1 showed a bias from the fictive nest direction both on the training and test fields (Fig. 3), we adjusted the target direction on the test field for each cluttered nest. The direction of the mean vector at the training site was coded as $90^\circ$. For the open nest, the fictive nest direction remained $90^\circ$. Bühlmann et al.’s (Bühlmann et al., 2011) results showed that this nest was oriented on average approximately in the fictive nest direction at both the training and test fields (see their Fig. 2). Fig. 4A shows that the two groups performed similarly on this dimension. The statistical comparisons between the cluttered nests and the open nest revealed neither a significant difference in mean direction (Watson-Williams test, $p = 0.891$), nor a significant difference in directional scatter (K-test, $p = 0.625$).

**Insert Fig. 4 about here**
Distances travelled

In terms of the distance travelled in the fictive nest direction at the start of search (the y-axis value, \(d_0\); Fig. 4b), ants of the cluttered nests displayed a significantly smaller variance than ants of the open nest (O’Brien’s test, \(F_{1,43} = 5.02, p = 0.030\)).

Welch’s ANOVA then showed that the ants from the open nest travelled farther before searching (\(F_{1,30.7} = 7.37, p = 0.011\)). The coefficient of variation (ratio of SD to M), however, was similar between the two groups (open nest: 0.49; cluttered nests: 0.47), replicating the linear scaling of standard deviations to means in odometry found in this species (Narendra et al., 2007), and also in *Cataglyphis fortis* (Cheng et al., 2006).

In total path length (\(l\), determined up to the point where searching began; Fig. 4c), the open- and cluttered-nest ants showed no significant difference in variance (O’Brien’s test, \(F_{1,43} = 2.20, p = 0.145\)). In means, the open-nest ants ran longer paths (\(F_{1,43} = 4.08, p = 0.050\)). Moreover, the variance in straightness (\(d/l\)) was much larger in the ants from the cluttered nests (Fig. 4d; O’Brien’s test, \(F_{1,43} = 9.60, p = 0.003\)). Furthermore, in means, the open-nest ants had straighter paths (Welch’s ANOVA, \(F_{1,32.2} = 8.62, p = 0.006\)).

Discussion

In a previous paper (Bühlmann et al., 2011; henceforth referred to as BCW) we showed that the open-habitat species *Cataglyphis fortis* relied more strongly on its path integration home vector when tested in the absence of familiar landmark cues than the cluttered-habitat species *Melophorus bagoti* did. The switch between vector-based and landmark-mediated behaviour occurred more readily in the latter than the former species. Across a range of conditions, *M. bagoti* switched from running off a vector to
searching, presumably for familiar landmarks, after a shorter distance than did *C. fortis*. We concluded that the open-habitat and cluttered-habitat species had higher and lower propensities, respectively, to adhere to their vector-based strategy when presented with unfamiliar landmark situations. Comparisons across past studies suggested that intraspecific, environment-dependent differences would be riding on these interspecific propensities found by Bühlmann et al. (Bühlmann et al., 2011). The present results provide further evidence by confirming this hypothesis within a single test situation in a single season.

Ants of the species *Melophorus bagoti* inhabiting either (exceptionally) an open-field habitat or (as usually) cluttered-field habitats were tested in comparison. Trained in their natural habitats to forage at a feeder placed at a 12-m distance from the nest, they had to perform their home runs in an open test field free of nest- or route-defining landmarks. Ants of both groups selected their home direction by relying on information provided by their path integrator. The angular spreads of their home runs did not differ between the two groups (Fig. 3a), as they did not differ between the two species *M. bagoti* and *C. fortis* (BCW, Fig. 5 therein). This behaviour indicates that the same path integration mechanism was used by ants of both species and environments. What did differ significantly, however, between the ants from the open nest and those from cluttered nests were (i) the beeline distances ($d_0$) they covered in the test field and (ii) the indices of straightness ($d/l$) of their trajectories. First, the beeline distance was significantly larger in the open-nest ants than in the cluttered-nest ants, meaning that the latter broke off their vector-based home runs much earlier than the former did (Fig. 3b). Note that Fig. 4b corresponds to Fig. 3 in BCW, blue boxplots [−/−] and [+/−], respectively. The [−/−] condition in BCW resembled our open-nest group, in that ants
from the same open nest were displaced to a test site on the balloon-launching field. The
[+/-] condition in BCW paralleled what was encountered by our cluttered-nest ants, in
that artificial route marks were set up in training, but then missing on tests. It seems that
the artificial landmark array with which *M. bagoti* had been presented in our former
study had about the same effect on the ants’ behaviour as the naturally cluttered
environment had in the present study. In each case, the missing landmarks resulted in
about 25% shorter runs before the start of search. Second, the home runs in the
cluttered-nest ants were distributed over a much larger area and thus exhibited smaller
indices of straightness, \( \frac{d}{l} \), than the home runs of the open-nest ants did (Fig. 3d).
Parametric manipulations on this species have shown that the larger the mismatch in
views between training and test situations, the more the ants tend to meander,
zigzagging around the direction of travel (Wystrach et al. 2011 and Fig. 5 therein). We
interpret this wiggling during travel as indicating a propensity to search for familiar
scenery even as the ants integrate a path.

We attribute the differences between the open and cluttered nests to the foraging
experience that the ants have had, in particular to the different visual input that they
have encountered in foraging on their respective terrains. We think it highly unlikely
that the open nest would have evolved different innate tendencies from those found in
the other nests in the vicinity. The balloon-launching field was only cleared very
recently in evolutionary time, within the last two centuries, and we doubt that adaptive
specialisations to such an environment would have evolved in that time.

The question arises as to how much experience of the visual surround is necessary
for the observed effect to appear, that is, for ants deprived of familiar landmarks to run a
shorter straight leg before engaging in searching? Indeed, even a couple of foraging
trips to food provided near the nest suffice to let the ant learn something of the visual panorama (Wystrach et al., 2012). In the context of the present study, the question is hard to test practically because ants make a good number of trips before arriving at a feeder 5 or 10 m away (personal observations), so that first-time arrivals at the feeder are not truly naïve. Wystrach et al. (Wystrach et al., 2012) conducted but did not report a condition in which ants that arrived at a feeder at the end of their experimental route for the first time were tested. But their behaviour was more similar to than different from the ants that were highly experienced with the route.

In sum, the results of the present study clearly show that differences in the readiness with which individual ants switch from vector-based to landmark-mediated behaviour depends on the landmark information which the ants have gained in their foraging area. The more cluttered the environment, the less strictly do conspecific ants rely on their path integration routine when deprived of familiar landmark cues. A species-specific, inherited propensity unravelled in BCW is further and finally shaped by environment-dependent, individually gained information.
References


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Figure captions

Fig. 1. Photographs of the habitats near Alice Springs Airport where the experimental nests were found. a) The open balloon-launching field where the open nest was found. b) and c) The sites where two of the cluttered nests were found. Photographs by RW (a) and EJTM (b, c).

Fig. 2. A few examples of the paths taken by tested ants from the open nest (a) and from the cluttered nests (b), obtained from the data sheets on which the paths were drawn. The red line indicate the start of the run to the point at which search began. Each square represents 1 m².

Fig. 3. Heading directions of ants from the cluttered nests at their training site (n = 10, 9, 10 for nest1, nest2, and nest3, respectively) and on the test field (n = 10 for each nest). At the training site, headings were taken after 15 s of travel from the feeder. On the test field, headings were taken at 80% of the path length to the point where searching behaviour began. 90° (thick horizontal line) indicates the feeder-nest direction. The graphs are actually circular plots, with values calculated according to circular statistics, but presented linearly because the overall scatter in heading was small.

Fig. 4. Comparing the performance of the ants from the open nest (n = 20) and from the cluttered nests (combined, n = 25) in a) heading direction, b) beeline distance to the nest at the point of first searching (d₀, the y-axis value at the point where searching first started), c) total path length (l) to the point where searching first started, and d) straightness (d/l), defined as the straight-line distance to the point where searching first started (d) divided by the total path length to that point (l). Heading directions in a) for the cluttered nests were calculated relative to the mean heading in the training area of
each nest (which equals 90°, the thick horizontal line), while the headings of open-nest
ants were not adjusted. The graph is a circular plot, with values calculated according to
circular statistics, but presented linearly because the overall scatter in direction was
small. The thick horizontal line in b) indicates the target distance. Significance levels: p
< 0.05 (*), p < 0.01 (**).
Fig. 4