

Learning, retention and coding of nest-associated visual cues by the Australian desert ant, *Melophorus bagoti*

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Abstract A variety of social insects use visual cues for homing. In this study, we examine the possible factors affecting the learning and retention of nest-associated visual cues by the Australian desert ant *Melophorus bagoti* and the manner in which such cues are encoded by foraging ants. We placed four prominent cylindrical landmarks around a nest and trained foragers from that nest to a food source. Ants were tested with the landmark array in a distant testing field after (1) a known number of exposures to the landmarks (1, 3, 7 or 15 trials, spread over a period of 1 day, 2 days or ≥ 3 days) and (2) after a known period of delay (0, 24, 48, 96 or 192 h). The results show that a combination of an increase in training trials and an increase in number of training days affected the acquisition of landmark memory. Moreover, once the landmarks were learnt, they became a part of long-term memory and lasted throughout the ants' foraging lifetime. To examine visual cue encoding behaviour, ants trained under similar conditions for 4 days were tested with (1) an identical landmark array, (2) landmarks of the same size used in

training, but placed at twice the distance from each other, and (3) landmarks whose dimensions were doubled and placed at twice the distance from each other. In conditions (1) and (3), the ants searched extensively at the centre of the four landmarks, suggesting that, similar to the Saharan ant (genus *Cataglyphis*) and the honeybee, *M. bagoti* too uses a snapshot to match the view of the landmarks around the nest. But contrary to the snapshot model, in condition (2), the ants did not search extensively at the centre of the landmarks, but searched primarily 0.5 m from the landmark, the distance from each landmark to the nest during training. We discuss how various search models fare in accounting for these findings.

Keywords Acquisition · Retention · Local vector · Snapshot · Landmarks · Desert ants · *Melophorus bagoti*

Introduction

An important feature of navigation for central place foraging insects is the requirement to return repeatedly to their nesting and feeding sites. To accomplish such a task, insects use a variety of strategies, from self-generated motion cues (Srinivasan et al. 1997) to guidance by visual landmarks (Wehner and R ber 1979). As the goal itself is often not visually distinct, its location may need to be encoded relative to nearby conspicuous cues. Ample evidence demonstrates that central place foragers, such as ants, bees, and wasps, use visually conspicuous objects to steer themselves towards the goal (Collett and Collett 2002; Wehner and R ber 1979). Visual cues not only provide pinpoint accuracy for individuals that are in the vicinity of the goal, but also act as a beacon, guiding insects from a long range (Cheng 2006; Collett 1995; Collett and Rees

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1997; Wehner et al. 1996). Some large-scale landmarks, such as panoramic skylines, may also be used in insect navigation, although this process is not well understood (Dyer and Gould 1983; Fukushi 2001; Menzel et al. 2005; von Frisch and Lindauer 1954).

Upon first leaving their nest, bees and wasps perform learning or orientation flights, which involve flying away from the nest in a series of arcs to learn the visual cues associated with it (Collett 1995; Lehrer 1993; Zeil 1993). After a few arrivals and departures, their flight paths become more direct, with only occasional turns to update their memory. Additionally, while leaving from a food source, wasps back away in a series of arcs roughly centred on the goal, enabling them to view the goal from a standard retinal position (Collett and Baron 1994; Collett and Rees 1997; Zeil et al. 1996). Similarly, ants engage in learning walks in which they turn and look back while returning from a feeding site (Nicholson et al. 1999). The frequency of such inspections is maximum while the ants are close to the food source and gradually reduces as the ants move away. It is during these learning walks that the visual cues are presumed to be learnt. Learning of these cues is typically rapid. Ants decrease the frequency of the backward turns as they become familiar with a particular feeding site after approximately three trips. Bees learn the visual features around the hive on their first orientation flight (Capaldi and Dyer 1999; Srinivasan et al. 1998), and ground-nesting wasps learn to associate new visual cues around their nest over a single flight (Tinbergen 1932).

One common way of using visual cues associated to a goal is to match the current retinotopic image to a previously encoded image, or a snapshot (ants: Åkesson and Wehner 2002; Durier et al. 2003; Judd and Collett 1998; Wehner and R ber 1979; Wehner et al. 1996; bees: Cartwright and Collett 1982, 1983; wasps: Zeil 1993). Honeybees learn the apparent size of the landmark and the compass direction associated with it (Cartwright and Collett 1983). Upon returning, they move to match the currently viewed image with the stored image. Ants take multiple snapshots (Graham et al. 2004; Judd and Collett 1998) and match them sequentially on the return journey.

Once learnt, visual cues associated to the nest are retained in the memory of the social insects for a relatively long time. Honeybees can return to their hive from a food source after a delay of 48 h (Uchida and Kuwabara 1951), while desert ants are able to use the memory of visual cues to home in to the nest even after a delay of 240 h (Ziegler and Wehner 1997). Some wood ants (*Formica rufa*) that have been isolated for up to 30 days have been observed to find a familiar route again (Rosengren 1971). In contrast, the memory of the distance travelled on an outbound trip is required only for the immediate return journey and, hence, decays after a delay of 24 h in desert ants (Cheng et al. 2006; Narendra et al. 2007).

In this study, we investigate the factors underlying the learning, retention and coding of the visual landmarks around the nest in the Australian desert ant *Melophorus bagoti*. These ants are solitary foragers and establish distinct individualistic routes between the nest and a food source (Kohler and Wehner 2005). They have a foraging half-life of 3.4 days and a life expectancy of ~4.9 days (Muser et al. 2005). Within such a short foraging life span, the ant has to learn the landmarks around the nest to pinpoint the location of the nest from close proximity. In this study, we first varied the number of training trials to examine the learning of nest-associated landmarks. We then varied the delay after the visual cues were learnt to study the retention of these cues in the ants' memory. We predicted that (a) learning of nest cues would be rapid, as returning to the nest at the end of each foraging trip is essential and (b) the memory of nest cues would be retained for an ant's foraging lifetime.

Finally, we manipulated landmark sizes and the distance between the landmarks to determine how nest-associated cues were encoded by the ants. We examine four models: (1) local vector and search, (2) contour model, (3) a bee snapshot model and (4) an ant snapshot model, to determine how they account for the ants' search patterns. We briefly explain below what each model predicts.

A local vector takes the ant from a recognised location (such as a landmark) in a particular direction for a particular distance (Collett et al. 1998). Ants displaced in an unfamiliar field may first head to one of the familiar (experimental) landmarks that acts as a beacon (Graham et al. 2003), then head towards the centre of the landmark array by relying on the local vector triggered by the familiar landmark and, finally, search systematically at the end of the vector. Such a model, consisting of a local vector plus search, predicts maximal searching at a fixed distance from the landmark. The contour model (M ller 2001) proposes that the ant abstracts a global parameter for matching, based on total contour lengths. A clear prediction of the model (and observed in *Cataglyphis* ants, Wehner and R ber 1979) is that, when both landmark distance and landmark size are doubled, the ants would continue to search at the centre. But when only the distance between landmarks is doubled, the ants would search close to the landmarks. When the landmarks are spread out, the total contour at the centre is too small (compared to training conditions), and being near a landmark increases the contour length on the eye. In the snapshot model proposed for bees (Cartwright and Collett 1982, 1983), the animal attempts to place the landmarks at the correct compass directions from the viewpoint of the target (nest). The model predicts that, in all tests, the animal should search most at the centre, because in each case the landmarks are at the correct compass directions from the vantage point of the centre.

Finally, in the snapshot model for ants, Nicholson et al. (1999) suggested that ants preferentially match what is in front of them. A possible reason is that ants must travel in the direction that they are facing and, unlike flying bees, cannot travel sideways. This model predicts that, if the landmark the ant heads to appears smaller than its counterpart in the stored snapshot, the ant would continue further, until the view of the landmark matches the earlier stored image. It then attempts to match the other landmarks. This model predicts extensive searching at the fictive nest site in the control condition and also when both landmark size and distance between landmarks are doubled, but searching should be dispersed when only the distance between landmarks is doubled.

Materials and methods

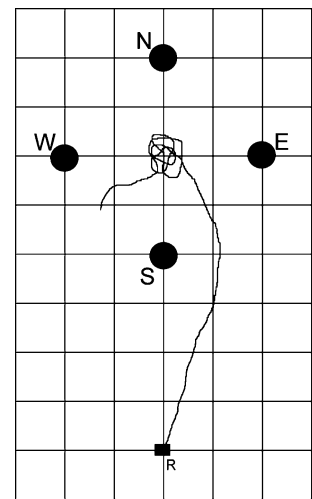
Study area and study species

The study was conducted in the semi-arid desert of Central Australia, 10 km south of Alice Springs, in the Northern Territory. The habitat was characterised by scattered tussocks of buffel grass, *Cenchrus ciliaris*, along with dispersed trees of the genera *Acacia* and *Hakea*. The desert ant *M. bagoti* is called *ituny ituny* by the local aboriginal people, which literally translates as ‘sun sun’, primarily because these ants are active only during the summer months from November to April. The ants begin foraging when the soil surface temperature rises above 50°C. Their typical foraging distance is around 20 m from the nest (Muser et al. 2005). The ants construct permanent nests (see Conway 1992 for nest information) and primarily scavenge for insects roasted in the desert heat.

Acquisition and retention experiments

Four black cylindrical landmarks measuring 1.5 m in height and 15 cm in diameter were placed vertically at a distance of 0.5 m from the focal nest in the four cardinal directions (Fig. 1). The landmarks were set up at the start of each day before the ants began foraging. A feeder was sunk into the ground due north 10 m away from the nest. Cookie crumbs and watermelon were provided to the ants as food. The interior walls of the feeder were coated with fluon to prevent the ants from escaping. Ants that reached the feeder and picked up a cookie crumb were marked for individual identification, using water-soluble acrylic paint. Marked ants were randomly allotted to different numbers of training trials in the acquisition experiment or to different delay periods in the retention experiment (see below). In both experiments, the first trip of the ant to the feeder, after which it was marked, was counted as ‘zero’; so, an

Fig. 1 Typical search pattern of a trained ant homing to the fictive nest located at the centre of the four cylinders (filled circles) in the test field. Ants were released at R. Grid size is 0.25×0.25 m



experimental ant was considered to have completed ‘one’ trip upon reaching the nest the second time. Ants of the one-training-trial group, therefore, would have typically seen the nest cues on four occasions: while leaving the nest (as unmarked individuals) on their ‘zero’ training trial, while returning to the nest after being marked, while leaving the nest as marked individuals on training trial ‘one’ and, finally, while returning to the nest at the end of that training trial.

During both acquisition and retention experiments, ants to be tested were captured just before entering the nest (<10 cm from the nest entrance) on their last training trial. As these ants had nearly completed their entire homing vector, we will refer to them as ‘zero-vector’ ants. Ants were collected in a plastic tube and transferred in the dark to a test field, which was located ~300 m away from the nest location and was unfamiliar to the ants. This ensured the absence of familiar distant cues, which the ants typically rely on for homing. The 5.0×5.0 m test field was divided into a grid of squares with sides of 0.5 m, using tent pegs and string. A landmark array identical to the one at the nest was set up. Strings were tightened and raised 3.0–5.0 cm above the surface of the ground to prevent ants from tripping over them. Zero-vector ants were released in the test field 1.5–1.75 m south of the fictive nest position. The experimenter then recorded the ant’s search path on grid paper for a 2-min period.

In the acquisition experiment, marked ants were randomly allotted to 1, 3, 7 or 15 training trials. As the entire training regime was normally not possible in a single day, the tested ants could also be grouped according to the number of days over which the training occurred, either 1, 2 or ≥3 days. On their last training trial, ants were captured close to the nest and immediately transferred in the dark to the test field where their paths were recorded. Care was taken to ensure that the ant did not drop its cookie crumb

during capture, thus ensuring the ants were motivated to search for their nest entrance when released in the test field.

In the retention experiment, ants were trained for seven to ten trials, with the training spread over at least two consecutive days for each individual ant. Data to be presented here show that ants learn the landmark array well with this training regime. In this study, too, zero-vector ants were captured and tested randomly at the specified delay period of 0, 24, 48, 96 or 192 h. Ants from the 0-h delay group were tested immediately, while ants allotted to the other groups were captured in plastic tubes and released after the specified delay period. During the delay period, ants were held individually under natural light–dark conditions and were provided with 10% honey–water solution as food. The mortality rate in captivity was approximately 5%. At the completion of the delay period, ants were provided with a cookie crumb and released in the test field.

Visual coding experiments

In this experiment, we examined how ants encode nest cues while homing in to the nest. The study was modelled after Wehner and R aber (1979). We studied four nests in total, but only one nest at any given time. Black cylindrical landmarks, measuring 60 cm in height and 16 cm in diameter, were erected at the four cardinal directions, 0.5 m from the nest entrance. A feeder was set up 6 m due south from the nest. Ants that arrived at the feeder on the first day were marked with three colour dots for individual identification and trained for four consecutive days to the feeder. Marked ants returning to the nest from the feeder on the fifth day were collected close to the nest and transferred to the test field.

The test field, a 5 m \times 5 m area, was \sim 300 m away from the nest location and was divided into a grid of squares (0.5 \times 0.5 m). Trained ants were tested under three test conditions, and each ant was tested only once. In the first condition, a landmark array similar to the training conditions (LD) was set up. In the second condition, the distance from the cylinder to the fictive nest was doubled from 0.5 to 1.0 m (LD⁺). In the third condition, not only was the distance from the landmark to the nest doubled, but the size of the landmarks was also doubled to 120 cm length and 32 cm diameter (L⁺D⁺). In the test field, ants were released individually, from four cardinal directions N, S, E and W, 2 m away from the fictive nest position. The paths of the released ants were recorded on grid paper for a 2-min period. For the analysis, we rotated the coordinate system in such a way that the release positions were superimposed. Of the four models discussed in “Introduction”, the local vector model predicts high searching at the fictive nest site in the control condition, LD, but maximum searching at a point midway between the landmark and the centre of the array in the LD⁺ and L⁺D⁺ conditions. The contour model predicts

high searching at the fictive nest site in LD and L⁺D⁺ conditions, while in the LD⁺ condition, search should be concentrated near the landmarks. The bee-snapshot model predicts high searching at the fictive nest in all three conditions. Finally, the ant-snapshot model predicts high searching at the fictive nest in the LD and L⁺D⁺ conditions, with no concentrated search in the LD⁺ condition.

Statistical analysis

The trajectories of the ants were digitised. Extensive searching at the fictive nest position would be reflected in high values of search density at the nest. The average search density was determined at every 25 cm² by counting the pixels in the square and normalising it as a proportion of the total number of pixels over the entire grid. An average of the search density at the four squares that surrounded the nest gave the search density at the nest for each ant.

In the acquisition experiment, the independent variables included ‘training trials’ and the ‘number of days’ over which the ants were trained. We determined if an increase in training trials, with the number of training days as a covariate, would result in higher search density at the fictive nest. The proportion of ants in each group that travelled within 10 cm of the nest was also determined. In the retention experiments, we determined if an increase in delay caused a decay in the ant’s visual memory of the nest cue. A decrease in search density at the nest with increasing delay would indicate a decay in the ant’s memory. In the visual coding experiments, the proportion of ants in each condition that travelled within 25 cm of the landmark closest to the release point was determined. The search density at the nest was compared between the three conditions, LD, LD⁺ and L⁺D⁺. Because of failure to meet assumptions of normality for parametric tests, the non-parametric Kruskal–Wallis test was used; this is a non-parametric analogue of a one-way analysis of variance (ANOVA). Dunnett’s test was used to compare conditions against the control condition. All statistics were carried out using the statistical package JMP (SAS 2002).

Results

Acquisition

In the test field, ants initially searched at the release point and then headed towards the fictive nest position located at the centre of the landmarks. The proportion of ants that reached 10 cm around the fictive nest site increased with experience (1 trial = 0.33, $N=11$; 3 trials = 0.54, $N=11$; 7 trials = 0.6, $N=20$ and 15 trials = 0.64, $N=17$). However, the search density at the fictive nest did not differ with increase

in the number of training trials (analysis of covariance [ANCOVA], $F=1.55$, $df=3$, $p=0.21$; Fig. 2). Suspecting that the large effect of the covariate might be playing a role, we then tested the data for a linear trend of increasing search density at the nest site with increasing numbers of training trials. The linear contrast turned out to be significant ($F=4.66$, $df=1$, $p<0.05$). A post hoc test comparing pairs of conditions revealed a significant difference in the search density at the nest only between the fifteen-trial and one-trial groups (Tukey–Kramer test, $p<0.05$).

We found that the search density at the fictive nest increased significantly with an increase in training days (ANCOVA, $F=12.42$, $df=1$, $p<0.001$; Table 1). The search density at the fictive nest did not differ between ants from the 1- and 2-day groups (Tukey–Kramer test, $p>0.05$). However, the search density differed significantly between 1- and ≥ 3 -day groups (Tukey–Kramer test, $p<0.05$) and 2- and ≥ 3 -day groups (Tukey–Kramer test, $p<0.05$). There

was no interaction between the number of training trials and training days (ANCOVA, $F=2.16$, $df=3$, $p>0.1$). These results need to be treated with some caution, because some cell numbers were low, and some were empty. This was because the effect of number of training days was unanticipated, and this factor was not experimentally controlled; the numbers of subjects were chosen to give sufficient power to test the effect of training trials. Nevertheless, the linear effect of training trials and a difference in search behaviour between ants trained on 3 or more days and those trained on fewer than 3 days (Table 1) both turned out to be significant.

Another measure of successful searching is the proportion of ants that would have found the nest had they not been displaced, defined as reaching within 10 cm of the fictive nest during a search (Table 1). These results parallel the measure of search density at the nest. The three highest proportions of ants that reached 10 cm around the fictive nest were from the

Fig. 2 Search density profiles of ants after different numbers of training trials. The fictive nest position *N* is at the centre of the four cylindrical landmarks (filled circles). Ants were released at *R*, 1.5 m away from the fictive nest position. Search patterns were superimposed on a common starting point, although the ants started at different locations in physical space. An area of 0.5×0.5 m (white square), around the nest, denotes the area where search density (means \pm SE) was measured. Grid size is 0.25×0.25 m

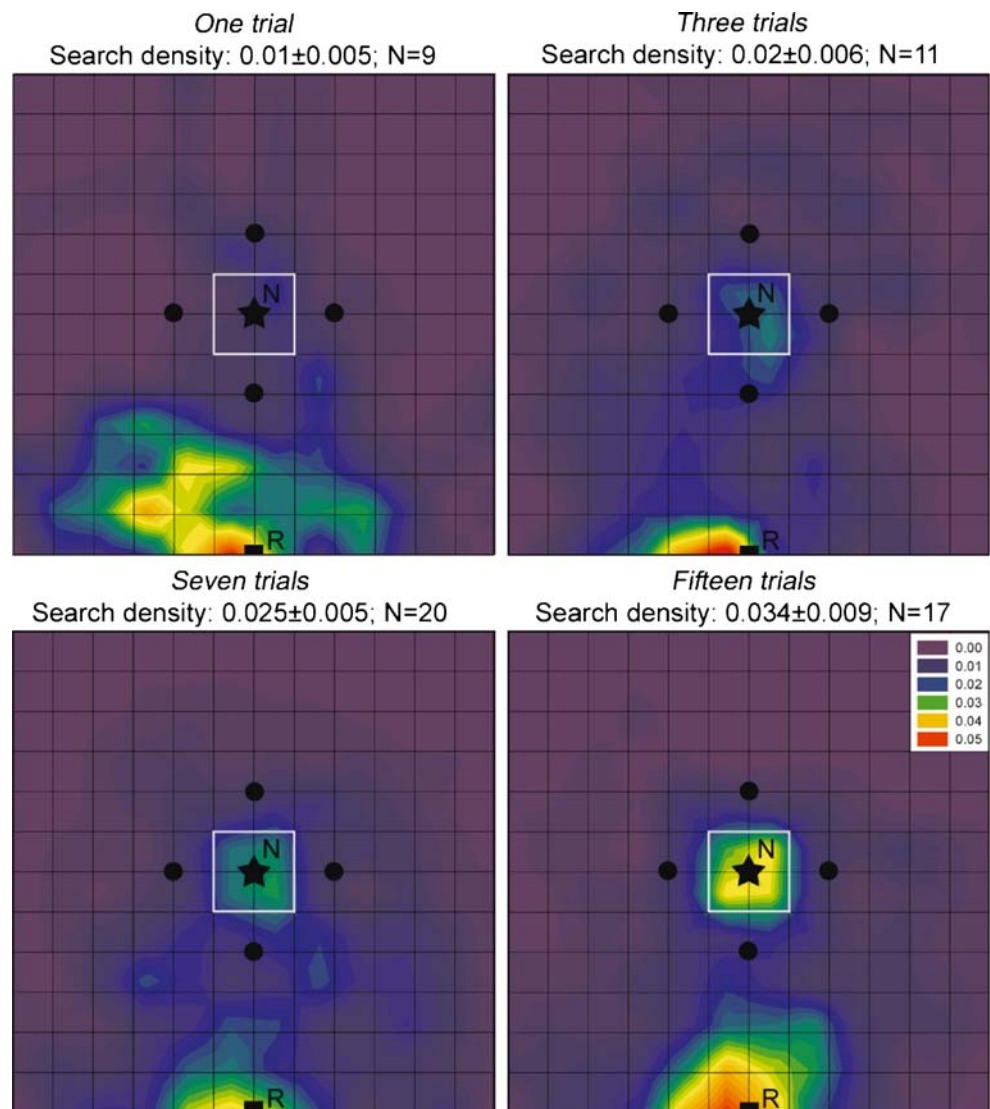


Table 1 Search density of ants at the fictive nest position (means±SE) with different numbers of training trials trained over different number of days

Training trials	1	3	7	15
1 day	0.010± 0.006 (N=7) <i>0.285</i>	0.008± 0.006 (N=5) <i>0.2</i>	0.009± 0.005 (N=6) <i>0.166</i>	–
2 days	–	0.014± 0.014 (N=2) <i>0.5</i>	0.022± 0.005 (N=10) <i>0.7</i>	0.003± 0.003 (N=4) <i>0.25</i>
≥3 days	0.007± 0.007 (N=2) <i>0.5</i>	0.036± 0.007 (N=4) <i>1.0</i>	0.051± 0.012 (N=4) <i>1.0</i>	0.042± 0.011 (N=13) <i>0.769</i>

Numbers in italics indicate the proportion of ants that got within 10 cm of the fictive nest.

groups: 3 trials + ≥3 days (1.0), 7 trials + ≥3 days (1.0) and 15 trials + ≥3 days (0.77). This raises the possibility of the requirement of sufficient number of both training trials and training days for learning visual nest associated cues.

Retention

To test the longevity of the landmark memory, we trained ants for seven to ten training trials over at least 2 days. After the training, ants were tested immediately or after different delay periods in a test field (Fig. 3). Ants from different delay groups did not exhibit any difference in the search density at the nest (one-way ANOVA, $F=0.42$, $df=4$, $p>0.1$). A post hoc test revealed no significant differences in the search densities between ants released immediately, i.e. the 0-h delay group and ants released after a delay of 24, 48, 96 or 192 h (Tukey–Kramer test, p 's>0.05).

Visual coding

Ants were trained to return to their nest, which was surrounded by conspicuous cylindrical landmarks (Fig. 4). Trained ants that were close to the nest were captured and released in a test field, where one of the following three landmark arrays were set up: a landmark array similar to the training condition (LD), an array in which the distance between the landmarks was doubled (LD⁺) and an array in which both the distance between the landmarks and the size of the landmarks were doubled (L⁺D⁺). The landmark closest to the release point sometimes acted as a beacon and attracted the test ants towards it (defined as reaching within 25 cm of the landmark). A majority of ants from the LD⁺

(0.71) and L⁺D⁺ (0.75) conditions were attracted to the landmark closest to the release point, after which they headed off towards the centre of the landmark array. In the LD condition, fewer ants (0.45) got within 25 cm of the landmark closest to the release point, as in this condition, the ants had to travel a slightly greater distance before reaching the landmark. Search density at the nest site differed significantly between the three conditions (Kruskal–Wallis test, $KW=29.86$, $p<0.001$). Search density of ants from the control group, LD, was similar to ants in L⁺D⁺ group (Fig. 4; Dunnett's test, $p>0.05$). However, the search density in the LD⁺ condition was significantly lower than both the LD (Dunnett's test, $p<0.001$) and L⁺D⁺ (Dunnett's test, $p<0.01$) conditions. Ants from the LD⁺ group searched 0.5 m away from the landmarks, but the search was short and not extensive (Fig. 5). Thus, the ants searched at the centre of the landmark array when both landmark distance and landmark size were doubled, but when only the landmark distance was doubled, ants briefly searched at the position corresponding to the distance from the landmark to the nest in the training situation.

Discussion

In the present account, we have shown that the acquisition of visual cues in *M. bagoti* ants is gradual. Ants with a single training trial did not learn to use the visual nest cues. A combination of several training trials and multiple days of training seems to be required for learning. Once the ants had learnt the visual cues, the memory of the cues did not decay even after an 8-day delay; thus, it lasted the ant's entire foraging life. In the visual coding experiments, ants searched extensively at the centre of the landmark array in both the LD and L⁺D⁺ conditions. Increasing only the distance between the landmarks without changing landmark size (LD⁺) resulted in a short search centred at the locations 0.5 m away from a landmark, the distance between landmarks and nest in the training situation.

The most interesting finding of this study is the slow and gradual acquisition of visual information with increase in training trials in *M. bagoti*. The search density around the fictive nest position gradually increased from 1, 3, 7 and 15 trials, but only the 15-trial group differed significantly from the 1-trial group (Fig. 2). This was surprising, as other hymenopterans, bees and wasps learn visual cues in a single training trial. Naïve bees home in to their hive from a distance of several metres by matching the view around the hive after a single learning flight (Capaldi and Dyer 1999; Srinivasan et al. 1998). Similarly, solitary wasps, *Philanthus triangulum*, learn visual nest cues (for example, pine cones arranged around their nest) in a single trial (Tinbergen 1932). This was demonstrated with a method

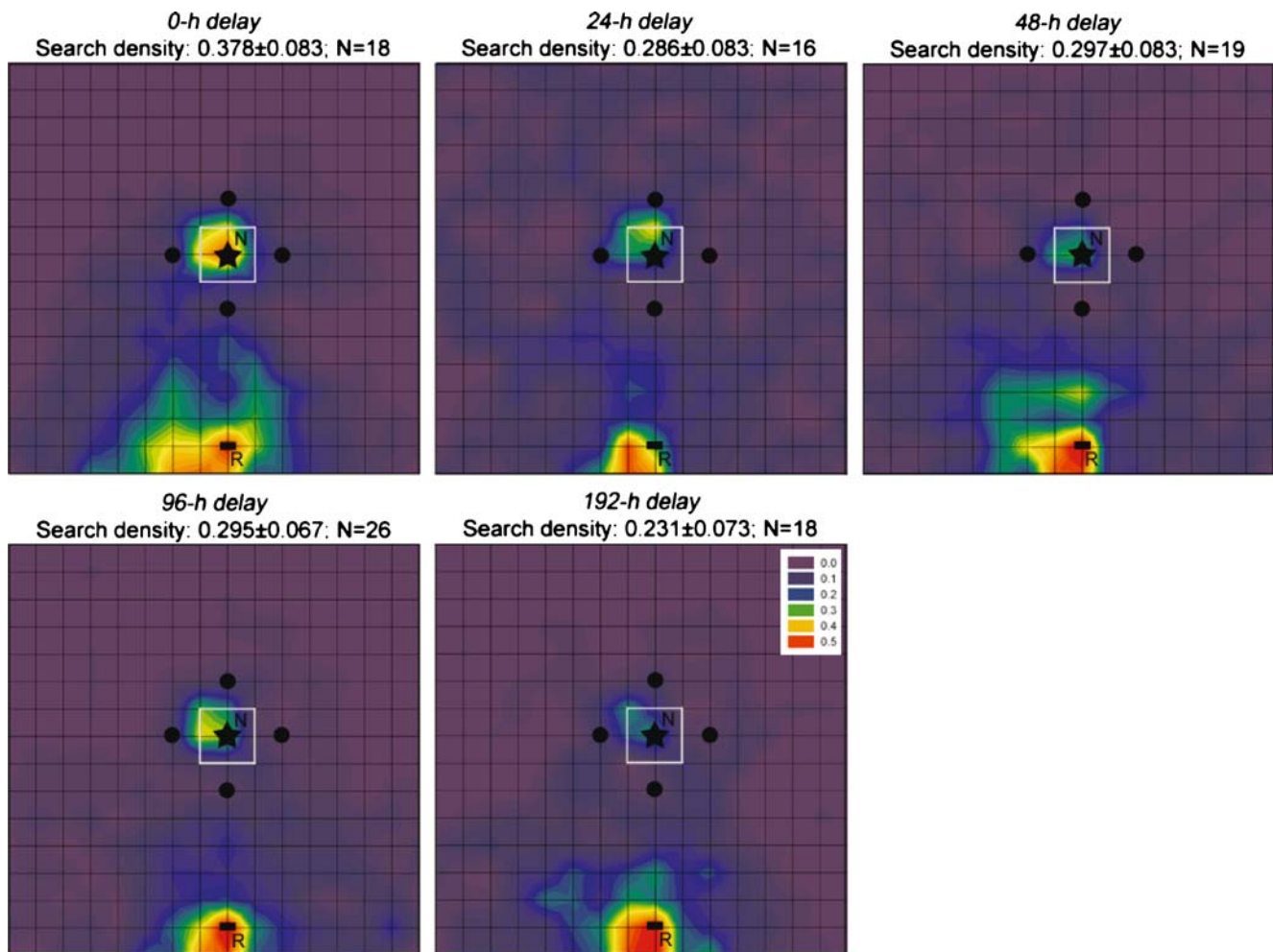


Fig. 3 Search density profiles of trained ants released following different delays. The fictive nest position *N* is at the centre of the four cylinders (filled circle). Ants were released at *R*, 1.75 m away from the fictive nest position. Search patterns were superimposed on a common

starting point, although the ants started at different locations in physical space. An area of 0.5×0.5 m (white square), around the nest, denotes the area where search density (means \pm SE) was measured. Grid size is 0.25×0.25 m

similar to the one used in the current study, namely by displacing the previously learnt cues to a nearby area, resulting in a returning wasp searching at a fictive nest site based on the newly set up array.

The slow and gradual acquisition of visual cues in *M. bagoti* ants is remarkably different from the rapid acquisition that occurs in other insects (Collett 1998). One reason behind this slow acquisition of visual nest cues may be ‘cue blocking’. Blocking of cues occurs when an earlier learnt element of a compound cue retards or blocks the learning of a new element of the compound cue (Kamin 1969). Learning A in phase 1 retards the learning of B presented in an AB compound in phase 2. Blocking would impair or reduce the learning of the new landmarks, as found in rats (Chamizo 2003) and bees (Cheng and Spetch 2001). Ants in our study were not naïve foragers and could have acquired and learnt other visual nest cues during their previous foraging trips. Hence, during the initial acquisition

trials, any natural landmarks the ants may have been using as cues might have blocked the learning of the new experimentally provided landmarks, causing the comparatively slow acquisition observed.

Although the search density at the nest and the proportion of ants that searched close to the nest (Table 1) gradually increased from 1–15 training trials, no significant difference in search density was found across the number of training trials. This could be due to the confounding factor of multiple training days (a factor covaried in the ANCOVA). Not all experimental ants could be trained and tested on a single day, and consequently, the training trials were spread over 1, 2 or ≥ 3 days. Although the number of training days was not independent of the number of training trials, the analyses suggest that multiple days of training played a significant role in the acquisition of visual nest cues. This conclusion is supported by studies on wood ants returning from a food source that had been experi-

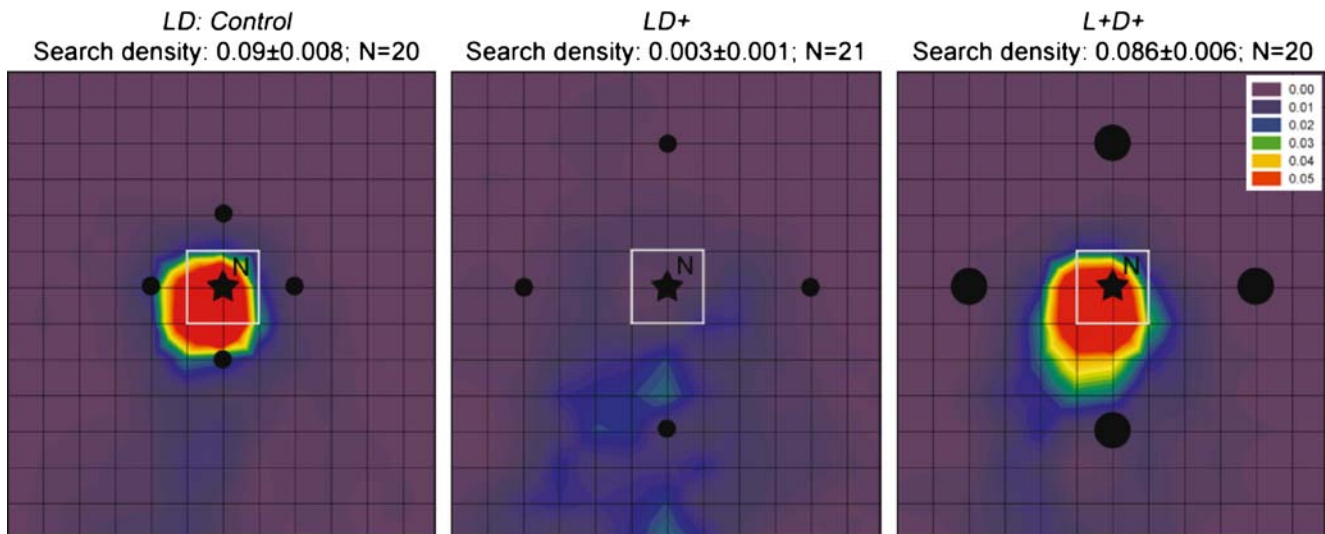


Fig. 4 Search density profiles of trained ants tested with different landmark configurations. The fictive nest position *N* is at the centre of the four cylinders (filled circle). The release point is 2 m away from the fictive nest and not shown here. Search patterns were superimposed on

a common starting point, although the ants started at different locations in physical space. An area of 0.5×0.5 m (white square), around the nest, denotes the area where search density (means \pm SE) was measured. Grid size is 0.25×0.25 m

mentally associated with new visual cues (Nicholson et al. 1999). The number of backward turns was used as a measure of the need to learn a new cue. As expected, these turns were maximum during the ant's first return trip from the feeder and gradually decreased with increase in training. The number of turns decreased over successive days, but interestingly, on their first departure on days 2 and 3, ants 'turned back and looked' an increasing number of times. The reappearance of turns on the first trial of the second and the third day supports the necessity of sighting a visual cue on multiple days for acquisition to occur.

Why would the number of training days matter in the acquisition of visual nest cues? Landmark stability and reliability is one possible reason why visual cues may need to be sighted on multiple days before they are acquired. *M. bagoti* ants inhabit a cluttered, tussock-rich habitat, where clumps of uprooted vegetation are often blown around amongst stable rooted plants. It would be costly to learn and rely on such unstable cues, which may be visually indistinguishable from their more stable and reliable counterparts. One way to increase the chance of using only reliable landmarks as cues would be to begin using a landmark as a cue only if it had been sighted on multiple days. Another factor that may have slowed acquisition is the rapidity with which these ants travelled between their nest and the feeder. Upon finding a food-rich zone, an ant's typical homeward and outward runs are extremely rapid, with most ants spending less than 20 s inside the nest to deposit the collected food (personal observation: Aung Si, Ajay Narendra) before they are out again on their next foraging trip. Such rapid excursions might also reduce the learning of the nest cues. Reluctance to rely on possible

unstable cues, along with the reduced opportunity to learn from rapid excursions, might have slowed the acquisition of the experimental landmarks. A controlled experiment is required to fully understand the effect of multiple training days. In our study, the number of training trials was the controlled factor, while the number of training days was not controlled (Table 1).

In the retention experiments, we predicted no deterioration of memory with increasing delay. True to the predictions, an increase in delay did not lead to any difference in the ant's search density at the fictive nest (Fig. 3). A similar high search density at the fictive nest exhibited by ants in the different delay groups showed that a training regime of seven to ten trials spread over at least 2 days was sufficient for acquiring visual nest cues. Visual cues associated with the nest were retained for the entire foraging lifetime by Saharan desert ants as well (Ziegler and Wehner 1997). Ants trained to a landmark configuration around the nest retained the memory of these landmarks for 20 days and searched extensively at the fictive nest location.

The final stage of homing involves using the landmarks around the nest. Landmark cues close to the nest are robust in pinpointing the goal, as even a slight movement by the ant would alter the appearance and the relative positions of the landmarks on the retina. If the ants extracted the total contour length for matching, as suggested by the contour model (Möller 2001), the search density around the landmarks in the LD⁺ condition should be high. This was not the case. The bee snapshot models refer to a class of template matching models, wherein the animal attempts to match what is currently perceived to an earlier stored retinotopic image (Zeil et al. 2003). Two influential models

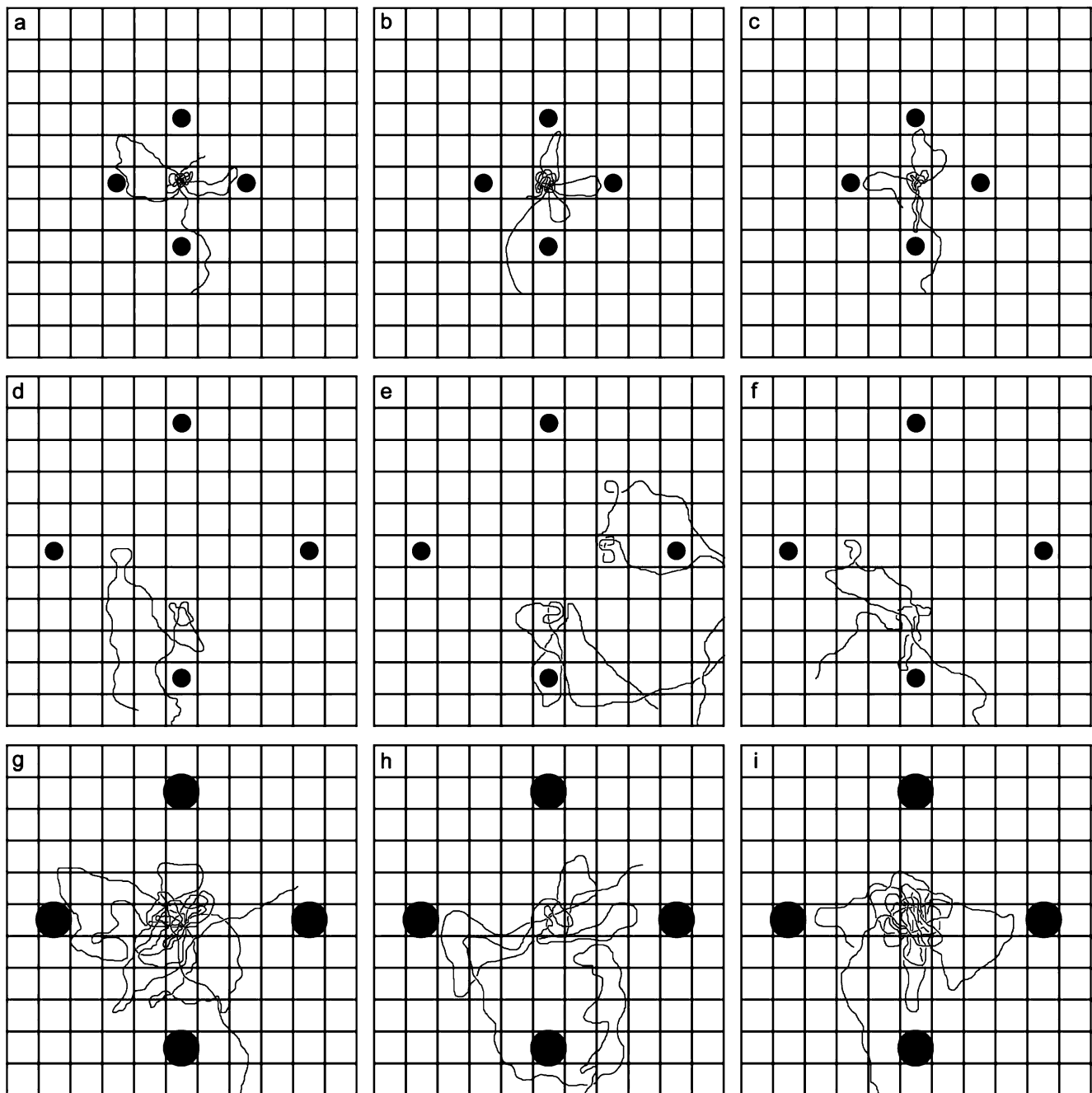


Fig. 5 Example paths of search patterns of ants that were tested in LD (a–c), LD⁺ (d–f) and L⁺D⁺ (g–i) conditions. The fictive nest is located at the centre of the array of the cylinders (*filled circles*). The release

point is 2 m south of the centre of the array and not shown here. Grid size is 0.25×0.25 m

were proposed by Cartwright and Collett (1982, 1983) to account for the performance of honeybees. They proposed that the bees learn the apparent size of each landmark and the compass direction to each landmark during training and move to match the current view with these specifications. As long as most of the landmarks on a test were in the correct direction from the vantage point of the fictive target, the bees found the fictive target and searched extensively at that position. The scattered searching of the ants in the LD⁺

condition, in which all the landmarks were in the correct compass directions from the vantage point of the centre of the array, is inconsistent with the bee snapshot model. If the ants used a local vector along with a systematic search, then the search density in the LD⁺ and L⁺D⁺ conditions should be concentrated at a fixed distance of 0.5 m from each landmark. In the LD⁺ condition, the ants did show a tendency to search, albeit briefly, 0.5 m away from each landmark, but this did not occur when landmark size and

the distance between landmarks were both doubled (L^+D^+ condition). The ant snapshot model proposed by Nicholson et al. (1999) predicts extensive searching at the nest site in the L^+D^+ condition and dispersed searching in the LD^+ condition. This was found in our study, and hence, this model best fits the data.

In the model proposed by Nicholson et al. (1999), the searcher has a tendency to move between the locations at which one of the landmarks matches its counterpart in the snapshot in direction and in apparent size. In the LD^+ condition, we observed a pattern of search behaviour in *M. bagoti* ants that does not fit the ant snapshot model. The search was brief but was always 50 cm away from the landmark at which the ant started its trajectory towards the fictive nest, a location at which the ant would have found the nest during the training situation (Fig. 5d–f). According to the model, however, the ant should have travelled farther, until the diagonally opposite landmark projected the correct retinal size (see Fig. 7E in Nicholson et al. 1999). Our interpretation is that, for an ant travelling from the landmark towards the centre of the array in the LD^+ condition (a travel distance of 1.0 m), the successive views would not match those encountered during training. The diagonally opposite landmark would appear too small, and the other two landmarks would also be in the wrong directions in addition to being too small. As the partial image matching strategy was not sufficient in this condition, the ants seemed to rely on the local vector triggered by the landmark, travelling 50 cm towards the centre of the array. But in searching at the end of the local vector, the ants did not find a good match, and they soon abandoned the search and returned to the same or to a different landmark to home in again (Fig. 5e). In contrast, this behaviour was not found in the L^+D^+ condition (Fig. 5g–i). In the L^+D^+ condition, as the ant travelled from any landmark towards the centre of the array, the successive views would match those encountered during training. A detailed model of the interaction between the local vector and the image matching strategy remains to be specified, and more data from different test conditions are needed to constrain modelling.

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