Acquiring, retaining and integrating memories of the outbound distance in the Australian desert ant *Melophorus bagoti*

Ajay Narendra[1,2,*], Ken Cheng[2] and Rüdiger Wehner[3]

[1]*Department of Biological Sciences and* [2]*Centre for the Integrative Study of Animal Behaviour, Macquarie University, Sydney, NSW 2109, Australia and* [3]*Department of Zoology, University of Zurich, Zurich, Switzerland*

*Author for correspondence at present address: Centre for Visual Sciences, Research School of Biological Sciences, Australian National University, PO Box 475, Biology Place, Canberra, ACT 2601, Australia (e-mail: ajay.narendra@gmail.com)

Summary

Estimation of distance travelled (odometry) forms a vital part of navigation for solitarily foraging ants. In this study we investigated the properties of odometric memory in the Australian desert ant *Melophorus bagoti*. Ants were trained to travel in linear channels to a feeder placed at 6 m or 12 m from the nest. We determined if the ability to estimate distances accurately increased with experience. We also determined the delay at which the odometric memory started to decay at both these distances. Ants with six trials of experience did not get better at estimating distances and the odometric memory decayed after a 24 h delay. We then determined if ants integrated their latest odometric memory with their previous memories. We did this by training two groups of ants for five trials, one group to 6 m and another to 12 m, and then halving or doubling the outbound distance on their sixth trip, respectively. The ant's estimate was noted when the ants were released either immediately or after a 24 h delay. Ants always estimated their last outbound distance when released immediately. However, they switched to route-based navigation rather than estimating distances after a 24 h delay (at which time their odometric memory would have decayed).

Key words: ant, distance estimation, acquisition, memory decay, integration, navigation, *Melophorus bagoti*.

Introduction

Ants, one of the most abundant groups of insects, exhibit diverse navigational strategies. Most of the commonly seen ants adhere to pheromones and reach the nest or the food source by forming long trails. Naïve foragers of some species of ants adhere to established trails when travelling to a food source (Breed et al., 1987). Experienced ants, however, switch to a faster means of navigation by using visual cues (Harrison et al., 1989). Pheromone trails are substituted by visual or self-generated motion cues in solitary foraging ants. Solitary foraging desert ants usually rely on path integration to home in to their nest. In path integration, an outward travelling ant keeps track of the distance and direction travelled from its starting point to compute the shortest return distance. In featureless landscapes such as salt pans, desert ants of the genus *Cataglyphis* use path integration alone to reach the nest, but also use landmark based route navigation when visual cues are available (Wehner, 2003). A successful foraging trip by a desert ant leads to repeated visits to the same foraging area (Wehner et al., 1983; Wehner et al., 2004). In another solitary foraging desert ant, *Melophorus bagoti*, each individual ant forages in a particular sector, the distance travelled within this sector increasing with experience (Muser

et al., 2005). This results in ants learning the landmarks within their foraging sector. As path integration is limited in precision (Müller and Wehner, 1988; Merkle et al., 2006), when sufficient route marks are available, ants establish idiosyncratic paths that meander around familiar tussocks in the desert environment (Kohler and Wehner, 2005). To establish such paths ants need to learn and memorize sequences of landmarks.

Path integration, the primary means of navigation for desert ants over featureless terrain, has been well studied in Saharan desert ants. These ants compute the shortest homebound vector, rather than retracing their circuitous outbound path, to reach the nest (Wehner and Wehner, 1990). The mechanism involves estimating distances and determining directions. Celestial cues based largely on polarized light are known to provide the directional cue to the ants (Wehner, 1994), while cues derived from the legs (some form of step counting) provide the ants an estimate of the distance travelled (Wittlinger et al., 2006). Very little is known about the memory properties of distance estimation. Experiments by Collett et al. revealed that *Cataglyphis* ants rely predominantly on their last outbound trip when estimating distance for path integration (Collett et al., 2003). Recently, we studied the acquisition, retention, and

integration of odometric memories in *C. fortis* (Cheng et al., 2006), an ant whose primary means of navigation is path integration. In acquisition, the ants were good at odometry from the start. Six trials of practice did not improve performance over one trial of training. Odometric memory, however, showed decay following a 24 h delay, in contrast to the lifelong retention of memories of landmarks (Wehner, 1981; Ziegler and Wehner, 1997). The ants also did not integrate multiple odometric memories, basing their odometric estimates solely on the last outbound trip.

In the present study we ask similar questions of acquisition, retention, and integration of odometric memories in *Melophorus bagoti*, a desert ant whose primary means of navigation is route following. This ant inhabits the arid ecosystems of Central Australia, which is typically dotted with low scrubs and trees, forming a landmark-rich habitat. The ant typically establishes and adheres to familiar routes interwoven around tussocks (Kohler and Wehner, 2005; Wehner et al., 2006). However, when familiar cues are absent and when forced to navigate by estimating distances, *M. bagoti* path integrates (Wehner et al., 2006) and estimates distances accurately (A. Narendra, manuscript submitted). Despite the differences in the habitat and navigational strategies, path integration, when used, serves a similar function in both *M. bagoti* and *C. fortis*. We here compare the functioning of the odometric memories in the Australian desert ant with that in the Saharan desert ant. We predict: (a) rapid acquisition with no effect of extended training, (b) decay of distance memories after a 24 h delay, and (c) no integration over multiple distance memories, the odometric estimate being based solely on the last outbound distance travelled.

Materials and methods
Study site and study species

We studied two nests of the thermophilic ant, *Melophorus bagoti* Lubbock, in its natural semi arid desert habitat in central Australia (Christian and Morton, 1992). The study site was located 10 km south of Alice Springs, Northern Territory, Australia. The habitat was characteristic of sandy clayey loamy soil with the ground cover dominated by Buffel grass *Cenchrus ciliaris* and overstory by *Acacia estrophiolata* and *Hakea eyreana*. *M. bagoti* are solitary foraging ants, walk at over 25 cm s^{-1}, and forage even when surface temperatures exceed 70°C. Most foraging runs are <1 h in duration and <20 m in length (Muser et al., 2005). *M. bagoti* ants feed on elaiosomes of *Acacia* seeds (A. Narendra, unpublished observations), but are primarily scavengers feeding on insects roasted in the desert heat, the majority being hymenopterans. They are polymorphic with a special morph called 'repletes' or 'honeypots' that acts as storage vessels to provide the colony with food while they aestivate in the winter (Conway, 1992).

Experimental set-up

We experimented on one nest at a given time. A rectangular plastic frame measuring 70 cm by 46 cm was placed with the nest in the centre of the frame (Fig. 1). The frame had three
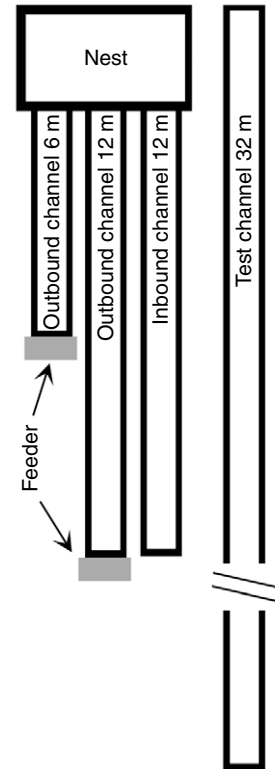

Fig. 1. Experimental set-up. The nest was enclosed from all sides, with two outbound channels of 6 m and 12 m leading to feeders sunk into the ground and one inbound channel connected to the enclosure. Plastic gates between the channels and the nest enclosure allowed control over entry and exit of ants. Ants that arrived at the feeder were picked up and released in the 32 m long test channel that was placed parallel to the outbound channels. Figure is not drawn to scale.

slots on one side to which plastic channels were connected. Two of the channels were outbound channels measuring 6 m and 12 m in length while the third was an inbound channel whose length was altered to 6 m or 12 m depending on the experiment in progress. Channels were constructed with 1 m segments (10 cm height and 10 cm width), joined to one another by nuts and bolts. A feeder was dug into the ground at the end of the outbound channel. Crumbs of cookies were provided as food for the ants. Sliding gates were used to control the entry and exit of ants in and out of the channels. A test channel of 32 m length was placed parallel to the other channels. A measuring tape was placed along the entire length of the test channel, enabling the observer to read off the distance travelled by ants.

General procedure

Ants reached a feeder by walking in one of the outbound channels. Ants that were tested immediately without any training were marked after the test (to ensure they were not tested again). The other ants were marked upon reaching the feeder for individual identification. After they had picked up a crumb of cookie, they were released in the inbound channel.

The sliding gate on the far end of the inbound channel was lifted to let the ant into the nest. On its subsequent trip, the ant was picked up in a plastic vial after it had picked up a cookie crumb and was either released in the inbound channel for further training or was taken for a test. Ants were transferred in the dark to a test channel where they were released 2 m from the far end of the channel (Fig. 1). The distance travelled by the ants from the point of release to the point of its first turn (start of search) provides an ant's estimate of the homebound distance. The point where the ant turns and walks back by at least 20 cm was identified as the first turn (Ronacher et al., 2000; Cheng and Wehner, 2002; Cheng et al., 2006). First turns were read off from the measuring tape to an accuracy of 0.1 m.

Acquisition: do ants estimate distances better with practice?

We asked whether ants with more experience would estimate distances better. We randomly assigned ants to two groups, one trained to 6 m and another to 12 m. Within each of this group, one batch of ants received zero training trial, another batch received one training trial, while another batch received six training trials. Zero-trial ants were those ants that reached the feeder for the first time, following which they were immediately tested. Ants of the one-trial batch were tested upon their second visit to the feeder, while ants from the six-trial batch were tested on the ant's seventh visit to the feeder.

Retention: does the ant's odometric memory decay over time?

The retention experiments were conducted to establish the delay at which odometric memory decay sets in. Sliding gates of both the 6 m and 12 m outbound channels were opened and ants were allowed to choose the exit. Ants that reached the feeder and picked up a cookie were collected in a plastic vial. One batch of ants were immediately tested to act as a control group, while others were released after a delay of 24 h, 48 h, 96 h or 192 h. Ants were individually held captive in natural light conditions in plastic tubes. Air, moisture, and food were available during captivity. Mortality rate in captivity averaged across different delay periods was 7%. Ants with different delay periods were released in the test channel, and their first turns were recorded. Besides an overall comparison of all delays, we explicitly evaluated 0 h and 24 h delay groups to test the hypothesis that memory decay is found after a 24 h delay.

Integration: do ants integrate two previously acquired distance memories?

We determined whether ants integrate previous odometric memories with their last outbound trip, when estimating distance immediately and after a delay of 24 h. We achieved this by training one batch of ants for five trials at 6 m and another batch for five trials at 12 m. On the sixth outbound trip, ants trained at 6 m had to travel twice their previously trained outbound distance, i.e. 12 m, to reach the feeder (6–12 group). On the other hand, ants trained at 12 m had to travel only half their previously trained outbound distance, i.e. 6 m, to reach a feeder (12–6 group) on their sixth outbound trip. Of the ants

that arrived at the feeder on their sixth trial, some were immediately released in the test channel and their first turns were determined. Some others were captured and kept in natural light conditions and released in the test channel after a 24 h delay. Their first turns were then recorded. Since there was no significant difference between zero training acquisition and zero delay retention groups (6 m: $F_{1,48}$=1.76, P=0.19; 12 m: $F_{1,64}$=0.01, P=0.90), we pooled the data to compare them with the immediately tested ants to determine if integration was occurring.

Analysis

The key dependent variable was the distance of the first turn. Initially the means and variances at both distances across different conditions were computed in all the experiments. The means were compared by Welch's ANOVA using JMP (SAS, 2002), because the test is suitable for comparing groups with heterogeneity of variance. Since the data included outliers, we analysed the variance by O'Brien test using JMP (O'Brien, 1979). This test is robust against such outliers. We also compared the variance between groups by using the coefficient of variation (CV) for every group. CV was computed by dividing the first turns of individual ants by the mean of their group. This variable was compared in an earlier study on *C. fortis* (Cheng et al., 2006), and we justify its use in the Results section. As a preliminary analysis, we first looked for a nest effect in all the formal statistics conducted. None were found, and we will thus ignore the nest factor in the results, and pool the ants from both the nests.

Results

We first determined how best to compare variances across groups. We pooled three groups of ants that were tested without delay (acquisition: 0 training trials, retention: 0 h delay, integration: 0 h delay), at 6 m and 12 m outbound distances. For each of the six groups, we converted individual scores to CVs by dividing each individual first turn by the group mean. The standard deviation (s.d.) for the pooled 6 m CV values was 0.37; the corresponding value for the 12 m was 0.39. These two values did not differ significantly (O'Brien's $F_{1,149}$=0.082, P=0.77). Thus, as in *C. fortis* (Cheng et al., 2006), inter-individual s.d. in odometric estimates of *M. bagoti* scales with the mean distance estimated, a form of Weber's law. We thus compared CVs when comparing variances between groups.

Acquisition

We determined if ants improve in estimating distances with training by comparing ants with zero, one and six training trials that were trained to a feeder at a particular distance (Fig. 2). At 6 m training distance, distance estimates of ants with zero, one and six training trials differed significantly (Welch's ANOVA $F_{2,38.552}$=9.79, P<0.001), and the unsystematic scatter (CV) did not (O'Brien's $F_{2,66}$=2.07, P=0.135). The mean distance estimates of ants with zero training was the most accurate among the three training groups. At 12 m training distance,
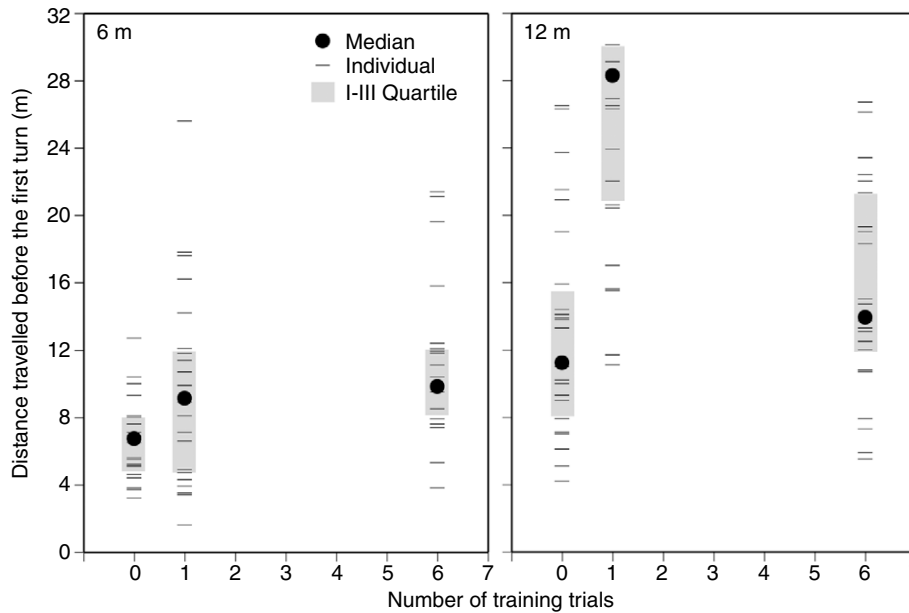
Fig. 2. Data from acquisition experiments. Distance estimates of ants trained to an outbound distance of 6 m or 12 m with zero (N=23, N=26, for 6 m and 12 m, respectively), one (N=23, N=26) and six (N=23, N=25) experiences. Individual first turns, medians, and quartiles are shown.

distance estimates of ants with zero, one and six training trials differed significantly (Welch's ANOVA $F_{2,49.314}$=25.91, P<0.001). Here too, ants with zero training were the most accurate among the three training groups. At 12 m training distance, the unsystematic scatter (CV) differed significantly (O'Brien's $F_{2,74}$=4.98, P=0.009), with the 1-trial group having the lowest variance in CV. It should be noted, however, that many ants in the 1-trial group ran close to the maximum distance. This resulted in a cluster of first turn distances near 30 m, artifactually reducing the variance. Given this artifact with the 1-trial group, we proceeded to compare the CVs of the 0-trial and 6-trial groups only to test whether performance improved with practice. The scatter of the two groups did not differ significantly (O'Brien's $F_{1,49}$=1.73, P=0.194).

Retention

Ants that arrived at a feeder at 6 m or 12 m distances were released in a test channel either immediately or after different delay periods. The mean distance estimates of ants (Fig. 3) gradually decreased as the delay period increased in the 6 m groups (Welch's ANOVA $F_{4,78}$=30.06, P<0.001), whereas there were no significant differences across groups in the unsystematic scatter (O'Brien's $F_{4,188}$=1.07, P=0.37). With increasing delay periods, first turns of ants with 12 m outbound distance decreased (Welch's ANOVA $F_{4,92}$=84.74, P<0.001), while the unsystematic scatter differed significantly across delay periods (O'Brien's $F_{4,199}$=2.52, P=0.04).

To test explicitly the hypothesis of decay after 24 h of delay, we compared only groups with 0 h and 24 h delays. The distance estimates differed significantly between the two delay groups at both training distances (6 m: Mann–Whitney U=314.5, P=0.002; 12 m: Mann–Whitney U=464.5, P=0.003).

Thus, odometric estimates were slightly shorter after a 24 h delay. CV values also differed between 0 h and 24 h delays (6 m: O'Brien's $F_{1,69}$=6.65, P=0.012; 12 m: O'Brien's $F_{1,76}$=5.24, P=0.025). Thus, the scatter was greater after a 24 h delay. The greater scatter in the 24 h delay groups might be caused at least in part by ants that were not path integrating. This is likely because some ants ran very short distances before turning back, resulting in the median first turn being shorter in the 24 h delay groups. We thus compared CV of control and 24 h delay ants again after excluding some ants from the lower end of the distribution in the 24 h groups. The ants with the shortest estimates were excluded until the mean first turns of the control and 24 h delay ants were as close to equal as possible. Even with this reduction in the variance of the 24 h delay groups, their CV still exceeded that of the control group (6 m: O'Brien's $F_{1,52}$=5.80, P=0.02; 12 m: O'Brien's $F_{1,67}$=15.03, P<0.001).

We also compared the odometric memory decay at 24 h between *M. bagoti* ants and *C. fortis* ants (from Cheng et al., 2006). To compare how memory decayed relative to controls (0 h delay), we adjusted the CV of *M. bagoti* ants by the ratio of the s.d. values of the two species at 0 h delay. Thus, CVs of *M. bagoti* at 24 h delay were each multiplied by the s.d. of CVs of *C. fortis* ants at 0 h delay and divided by the s.d. of CVs of *M. bagoti* ants at 0 h delay. The resulting comparison of the variances of adjusted CV values showed that they did not differ significantly between species at 6 m (O'Brien's $F_{1,63}$=2.34, P=0.131), but *M. bagoti* ants had greater variance at 12 m (O'Brien's $F_{1,60}$=5.70, P=0.02). Doing away with the adjustment still resulted in the same pattern of inferential statistics. Thus, the memory decayed more at 24 h in *M. bagoti* ants at 12 m outbound distance. We did not compare species at

longer delays because at those delays, both species started showing a substantial drop in their odometric estimates.

Integration

To determine if ants integrate memories of distance travelled, we trained (five trials) two groups of ants to a feeder, one at 6 m and another at 12 m. On their sixth trip to the feeder, the outbound distance of the 6 m trained ants was doubled to 12 m and the outbound distance for the 12 m trained ants was halved to 6 m. Ants that picked up the cookie were tested either immediately or after a 24 h delay. Ants of the 6 m group (Fig. 4), which experienced double the usual outbound distance on their last outbound trip, estimated their last outbound distance (13.85±6.27 m, mean ± s.d.) when tested immediately. The 12 m ant groups, which experienced half the usual outbound distance on their last outbound trip, also estimated their last outbound distance (6.63±1.62 m, mean ± s.d.) when tested immediately. After a day's delay, the distance estimates of ants that experienced doubling (24.73±7.08 m, mean ± s.d.) and halving (16.65±9.17 m, mean ± s.d.) on their last outbound distance overestimated their respective outbound distances. Many of the ants from the 12 m group ran the entire length of the channel when released after a 1-day delay.

We first compared distance estimates of ants tested immediately with the pooled estimates of ants from the zero retention and zero acquisition groups. The estimates of ants from the 6–12 group did not differ significantly from the 12 m outbound retention and acquisition groups (Welch's ANOVA $F_{1,22}=0.26$, $P=0.61$). There was no significant difference in their unsystematic scatter (CV) (O'Brien's $F_{1,82}=1.16$, $P=0.28$). The distance estimates of ants from the 12–6 group also did not differ significantly from its corresponding retention and acquisition groups (Welch's ANOVA $F_{1,49}=0.82$, $P=0.36$). There was no significant difference in their unsystematic scatter (O'Brien's $F_{1,65}=2.06$, $P=0.15$) too. Thus, the immediately tested animals did not integrate odometric memories; performance was based on the last outbound trip.

We then compared the immediately tested ants with those tested after a 24 h delay. A significant difference in mean first turn was found for the 12–6 groups (Welch's ANOVA $F_{1,17}=19.31$, $P<0.001$) and for the 6–12 groups (Welch's ANOVA $F_{1,31}=23.01$, $P<0.001$). The 0 h delay group had a significantly smaller CV than the 24 h delay group in the 12–6 groups (O'Brien's $F_{1,32}=11.29$, $P=0.002$), while in the 6–12 groups, CV did not differ between 0 h delay and 24 h delay groups (O'Brien's $F_{1,33}=2.11$, $P=0.15$). It should be noted, however, that many animals in the 24 h delay group ran close to the entire 30 m maximum distance (10 animals ran >26 m before turning); this means that the variance of this group was artifactually reduced.

Comparison of species in control conditions

Finally, we compared our data (Acquisition: 0 training trials, Retention: 0 h delay, and Integration: 0 h delay) from *M. bagoti* ants with *C. fortis* ants (Cheng et al., 2006). For each group in each species, CV was calculated relative to the mean of that
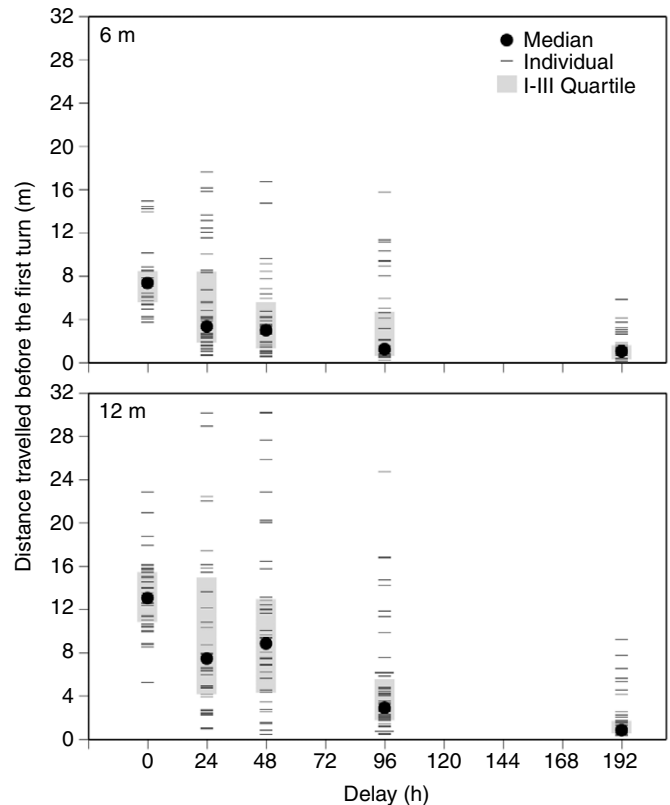

Fig. 3. Data from retention experiments. Distance estimates of ants released following a delay of 0 h ($N=27$, $N=40$, for 6 m and 12 m, respectively), 24 h ($N=42$, $N=38$), 48 h ($N=36$, $N=39$), 96 h ($N=42$, $N=46$), and 192 h ($N=43$, $N=41$), trained to an outbound distance of 6 m (top) and 12 m (bottom). Individual first turns, medians, and quartiles are shown.

group. We then compared the CV across species for all three conditions combined. Other conditions in the Acquisition experiments were not included because the *M. bagoti* ants overestimated in those conditions. At 6 m, the CV did not differ significantly between species in variance (O'Brien's $F_{1,121}=1.62$, $P=0.205$). At 12 m, *M. bagoti* ants had larger scatter in their CV than *C. fortis* ants (O'Brien's $F_{1,138}=7.37$, $P=0.008$). Thus, at longer distances, performance on odometric estimation in *M. bagoti* ants is inferior to *C. fortis* ants.

Discussion

The results of this study support the predictions made on functional grounds. We found that (a) although odometric estimates of ants with 0, 1 and 6 training trials differed significantly, performance did not get better with more training, (b) odometric memories decayed after a delay of 24 h, and (c) odometric estimates were based solely on the last (current) outbound trip.

Path integration functions to estimate the vector (distance and direction from the starting point, typically home) of the current journey. Being a lifeline when other navigation systems fail, path integration must be based solely on the current
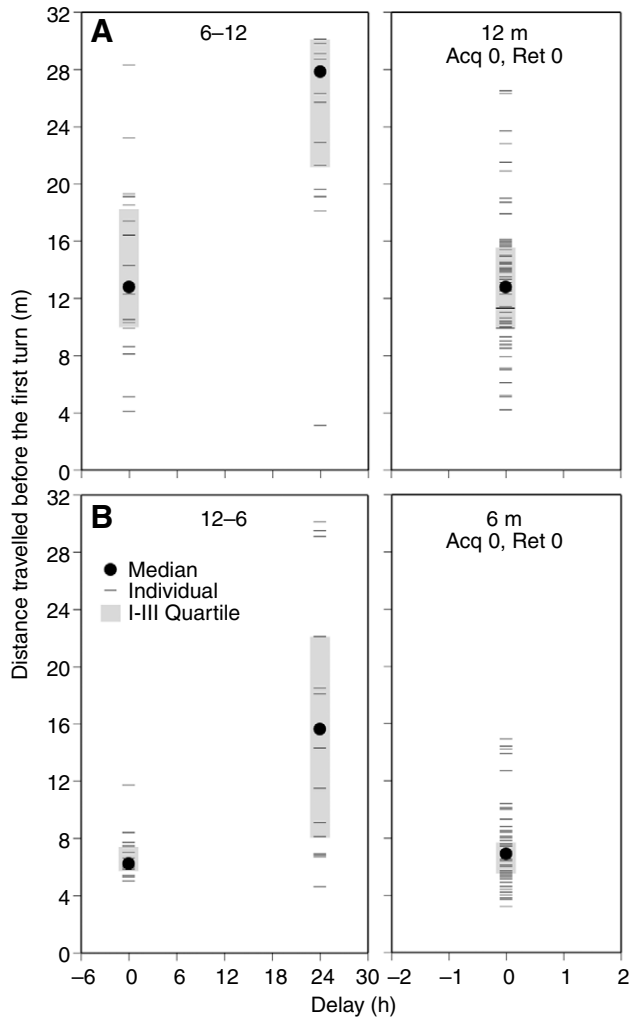
Fig. 4. Data from integration experiments. (A) Ants trained five times at 6-m outbound distance and tested after a 12 m outbound trip (6–12) either immediately (*N*=18) or after a delay of 24 h (*N*=17). (B) Ants trained five times at 12 m outbound distance and tested after a 6 m outbound trip (12–6) either immediately (*N*=17) or after a delay of 24 h (*N*=17). Individual first turns, medians, and quartiles are shown. Data from the 0 h delay retention (Ret 0) group and 0-trial acquisition (Acq 0) group were pooled and are shown for both 6 m (*N*=50) and 12 m (*N*=66) distances.

outbound trip, and not on previous outbound trips. Hence we predicted a lack of increase in accuracy in distance estimation with increase in the number of training trials. At both 6 m and 12 m training distance, ants with no training (0-trial group) were the most accurate in estimating the homebound distance. The average distances estimated by ants with zero training from the 6 m and 12 m group were greater by 0.56 m and 0.93 m than their respective true homebound distances. These mean values do not differ significantly from their respective outbound distances.

An increase in training trials, by either one or six, resulted in ants over estimating their true homebound distance (Fig. 2).

At 6 m training distance, the distance estimates of ants with one and six training trials were similar, but both greater than those of ants with no training. At 12 m training distance, 1-trial ants ran much farther than 0-trial ants, running on average a distance nearly twice that of 0-trial ants before turning back, while ants with six training trials estimated a distance 45% greater than ants with zero training. All groups of ants with training overestimated; the 95% confidence interval around the mean exceeded the outbound distance in each group.

A significant difference in the CV values between the training groups was found only at 12 m training distance. This was caused by the 1-trial group, which had a lower variance than the other groups; the 0-trial and 6-trial groups had similar variances. The lower variance in the 1-trial group was an artifact stemming from the fact that this group far overestimated the outbound distance, and many ants ran close to the maximum distance. The length of the test channel thus artifactually reduced the variance in this group. Overall, increase in training did not result in improved performance, confirming predictions.

We interpret the overestimation with training as an effect of learning to follow a route, an interpretation we also proposed for *C. fortis* (Cheng et al., 2006). When the ants run down the channel on a return journey, they learn to associate the context of the channel with a route instruction: run along the channel in the homebound direction until the nest enclosure is encountered. The view of the end of the channel, with the white walls of the enclosure at the end, might well form part of the route memory. Route following in both *C. fortis* (Wehner et al., 1996) and *M. bagoti* (Kohler and Wehner, 2005) is well known. Similar overestimation is found here in the integration experiment after a delay is imposed before the test (discussed below). Note that the ants generally did not run all the way down to the end of the channel. We interpret this to mean that the path integrator continues to operate in route following, a claim supported by evidence (Collett et al., 1998; Andel and Wehner, 2004; Knaden and Wehner, 2006). When executing a route, the ants still 'listen' to their odometer and turn back to search when they have travelled too far beyond. Further research would be required to confirm this interpretation.

Based on our previous study on *C. fortis* (Cheng et al., 2006), we expected an increase in scatter in odometric estimates of *M. bagoti* ants after a 24 h delay. Considering that the maximum foraging duration of an individual ant was 64 min (Muser et al., 2005), with most foraging trips being much shorter, there is no requirement for the odometric estimate to last beyond 24 h. As expected, ants released after a 24 h delay had increased scatter compared with the no-delay group (Fig. 3). This was the case even when mean first turn distance was equated between controls and 24 h delay groups by excluding ants in the 24 h delay groups with the lowest first turn distances. With other delays, the scatter was similar to the 24 h group; this no doubt caused the lack of statistically significant results in CV when all groups at 6 m outbound distance were tested together. The pattern of odometric memory decay in *M. bagoti* is similar to the Saharan desert ant, *C. fortis* (Ziegler and Wehner, 1997;

Cheng et al., 2006), which also showed an increase in scatter in their odometric estimates after a 1-day delay. At 12 m outbound distance, the decay in memory was greater in *M. bagoti* ants than in *C. fortis* ants (Cheng et al., 2006). This difference should be treated with caution because in the current study, ants with a 24 h delay ran a shorter distance on average before turning back. Some ants might have been engaging in systematic search from the start rather than estimating the outbound distance (discussed below). This would increase the variance for reasons other than memory decay. In *C. fortis*, a 24 h delay did not lead to significant changes in the average first turn distance (Cheng et al., 2006).

An increase in the delay to 4 days resulted in the mean first turns of *M. bagoti* decreasing. Ants tested after an 8-day delay ran on average <2 m before turning back. *C. fortis* behaved similarly after 8 days of delay (Ziegler and Wehner, 1997; Cheng et al., 2006). As with *C. fortis*, we interpret the very short runs in *M. bagoti* to indicate that the ants had abandoned path integration altogether, and turned instead to a search behaviour, usually exhibited by ants that have run off their entire global vector (Wehner and Srinivasan, 1981).

In integration experiments, ants based their odometric estimates solely on the last outbound trip. Ants travelled five times at one outbound distance, and on the sixth trip, the outbound distance was either doubled or halved. When immediately tested, the odometric estimates of the integration ants were similar to those without the previous training at a different outbound distance (0 h delay group: Retention experiment, 0-trial group: Acquisition experiment), confirming predictions. Other studies with ants (Collett et al., 2003) and bees (Lindauer, 1963) also show that odometric estimates are predominantly based on the current outbound trip. The predictions were not confirmed for ants tested after a 24 h delay; these ants ran much farther than the last outbound distance (Fig. 4), a pattern also found in *C. fortis* (Cheng et al., 2006). Many ants travelled the entire 30 m distance of the channel. Similar to acquisition data, we interpret the overestimation to indicate route-based navigation, including perhaps guidance by a remembered view of what the end of the channel looked like. This route-based navigation directs the ants to walk until they encounter the door that leads to their nest enclosure. Untrained ants did not exhibit this form of a route-based navigation. We suppose that the switch to route-based navigation occurs in ants because of a combination of memory decay and multiple training.

The lack of integration in path integration is understandable for functional reasons. The path integrator functions to compute the last outbound path and only that, and its output is thus not stored as a long-term memory (Collett et al., 2003). In contrast, landmark constellations or the profitability of food patches are stored in long-term memory (Wehner, 1981; Devenport et al., 1997; Ziegler and Wehner, 1997), and sometimes integrated. For example, Devenport et al. provided rats with two 'patches' (recessed food magazines separated in space) from which to retrieve food (Devenport et al., 1997). Patch A was most profitable in a first phase of training, while conditions reversed in a second phase and Patch B was most profitable. When tested immediately after Phase 2 training, rats preferred to visit Patch B, 'betting on' the most recent profitable patch. After a few hours of delay, however, the rats preferred whichever patch had been most profitable on average, showing that they remembered and compared all their past experiences with food in the experimental arena.

What do these findings imply for the ant in its natural habitat? The path of an ant's route is initially computed by the path integrator. With repeated experience along a path, cues along the route are used to set up route-based navigation (Kohler and Wehner, 2005; Wehner et al., 2006), with path integration as a back-up system. In their usual foraging grounds *M. bagoti* exhibits sector fidelity, with the foraging distance within the sector increasing with an ant's experience (Muser et al., 2005). This not only leaves the inexperienced workers to collect food closer to the nest, but also results in the experienced workers learning the routes, and familiarising themselves with landmarks they encounter along the route. This is reflected in the homebound paths of the ants. Rather than taking the direct route, ants adhere to stereotypical routes and wind around specific tussocks in similar ways over subsequent trips (Kohler and Wehner, 2005). Our findings in the channels are consistent with route learning. Odometric memory is a short-term memory as every outbound trip is unique for an ant. Ants with multiple experiences to a feeder do not learn the distance better, but familiarise themselves with the routes. Hence when trained to different distances they do not integrate distance memories but switch to a route-based strategy, following a decay in odometric memory.

References

Andel, D. and Wehner, R. (2004). Path integration in desert ants, *Cataglyphis*: how to make a homing ant run away from home. *Proc. R. Soc. Lond. B Biol. Sci.* **271**, 1485-1489.

Breed, M. D., Fewell, J. H., Moore, A. J. and Williams, K. R. (1987). Graded recruitment in a ponerine ant. *Behav. Ecol. Sociobiol.* **20**, 407-411.

Cheng, K. and Wehner, R. (2002). Navigating desert ants (*Cataglyphis fortis*) learn to alter their search patterns on their homebound journey. *Physiol. Entomol.* **27**, 285-290.

Cheng, K., Narendra, A. and Wehner, R. (2006). Behavioral ecology of odometric memories in desert ants: acquisition, retention and integration. *Behav. Ecol.* **17**, 227-235.

Christian, K. A. and Morton, S. R. (1992). Extreme thermophilia in a central Australian ant *Melophorus bagoti*. *Physiol. Zool.* **65**, 885-905.

Collett, M., Collett, T. S., Bisch, S. and Wehner, R. (1998). Local and global vectors in desert ant navigation. *Nature* **394**, 269-272.

Collett, M., Collett, T. S., Chameron, S. and Wehner, R. (2003). Do familiar landmarks reset the global path integration system of desert ants? *J. Exp. Biol.* **206**, 877-882.

Conway, J. R. (1992). Notes on the excavation of a nest of *Melophorus bagoti*

Lubbock in the Northern Territory, Australia (Hymenoptera:Formicidae). *J. Aust. Entomol. Soc.* **31**, 247-248.

Devenport, L., Hill, T., Wilson, M. and Ogden, E. (1997). Tracking and averaging in variable environments: a transition rule. *J. Exp. Psychol. Anim. Behav. Process* **23**, 450-460.

Harrison, J. F., Fewell, J. H., Stiller, T. M. and Breed, M. D. (1989). Effects of experience on use of orientation cues in the giant tropical ant. *Anim. Behav.* **37**, 869-871.

Knaden, M. and Wehner, R. (2006). Ant navigation: resetting the path integrator. *J. Exp. Biol.* **209**, 26-31.

Kohler, M. and Wehner, R. (2005). Idiosyncratic route-based memories in desert ants, *Melophorus bagoti*: how do they interact with path integration vectors? *Neurobiol. Learn. Mem.* **83**, 1-12.

Lindauer, M. (1963). Kompassorientierung. *Ergeb. Biol.* **26**, 158-181.

Merkle, T., Knaden, M. and Wehner, R. (2006). Uncertainty about nest position influences systematic search strategies in desert ants. *J. Exp. Biol.* **209**, 3545-3549.

Müller, M. and Wehner, R. (1988). Path integration in desert ants. *Proc. Natl. Acad. Sci. USA* **85**, 5287-5290.

Muser, B., Sommer, S., Wolf, H. and Wehner, R. (2005). Foraging ecology of the Australian desert ant *Melophorus bagoti. Aust. J. Zool.* **53**, 301-311.

O'Brien, R. G. (1979). A general ANOVA method for robust tests of additive models of variances. *J. Am. Stat. Assoc.* **74**, 877-880.

Ronacher, B., Gallizzi, K., Wohlgemuth, S. and Wehner, R. (2000). Lateral optic flow does not influence distance estimation in the desert ant *Cataglyphis fortis*. *J. Exp. Biol.* **203**, 1113-1121.

SAS (2002). *JMP Statistics and Graphics Guide, Version 5.* Cary, NC: SAS Institute.

Wehner, R. (1981). Spatial vision in arthropods. In *Handbook of Sensory Physiology*. Vol. VII/6c (ed. H. Autrum), pp. 288-616. Berlin, Heidelberg, New York: Springer.

Wehner, R. (1994). The polarization-vision project: championing organismic biology. In *Neural Basis of Behavioural Adaptation* (ed. K. Schildberger and N. Elsner), pp. 103-143. Stuttgart, New York: G. Fischer.

Wehner, R. (2003). Desert ant navigation: how miniature brains solve complex tasks. Karl von Frisch Lecture. *J. Comp. Physiol. A* **189**, 579-588.

Wehner, R. and Srinivasan, M. V. (1981). Searching behaviour of desert ants, genus *Cataglyphis* (Formicidae, Hymenoptera). *J. Comp. Physiol. A* **142**, 315-338.

Wehner, R. and Wehner, S. (1990). Insect navigation: use of maps or Ariadne's thread. *Ethol. Ecol. Evol.* **2**, 27-48.

Wehner, R., Harkness, R. D. and Schimd-Hempel, P. (1983). Foraging strategies in individually searching ants, *Cataglyphis bicolor* (Hymenoptera, Formicidae). Stuttgart: Fischer.

Wehner, R., Michel, B. and Antonsen, P. (1996). Visual navigation in insects: coupling of egocentric and geocentric information. *J. Exp. Biol.* **199**, 129-140.

Wehner, R., Meier, C. and Zollikofer, C. (2004). The ontogeny of foraging behaviour in desert ants, *Cataglyphis fortis. Ecol. Entomol.* **29**, 240-250.

Wehner, R., Boyer, M., Loertscher, F., Sommer, S. and Menzi, U. (2006). Ant navigation: one-way routes rather than maps. *Curr. Biol.* **16**, 75-79.

Wittlinger, M., Wehner, R. and Wolf, H. (2006). The ant odometer: stepping on stilts and stumps. *Science* **312**, 1965-1967.

Ziegler, P. and Wehner, R. (1997). Time-courses of memory decay in vector based and landmark-based systems of navigation in desert ants, *Cataglyphis fortis. J. Comp. Physiol. A* **181**, 13-20.