Supporting Information

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Additional Methods

Experiments were carried out with a single *Cataglyphis fortis* nest on the island of Djerba, Tunisia, during June 2008. The landscape was particularly flat and featureless, with no other prominent features within 30–200 m of the experimental area, depending on direction (Fig. S1). Ants were trained to a feeder (a slice of watermelon) 14 m North from their nest. A black cylinder 70 cm high and 40 cm wide was placed at the midpoint, but 1.5 m to the side, of the direct line between the nest and feeder. Initial training to the feeder was effected by moving a piece of watermelon incrementally, in steps of 1 m, along the direct line from nest to feeder. Both landmark and feeder were removed overnight and replaced each morning. Fine string was laid out in a grid of 1-m squares across the area so that the trajectories of ants could be followed and transcribed to squared paper with an accuracy of ≈10 cm.

Approximately 80–120 ants were at the feeder at any time, suggesting that the total number of foragers visiting the feeder was 250–300. An ant to be tested was selected at random from those preparing to leave the melon. The selected ant was given a crumb of biscuit to ensure a continued homeward motivation, and to allow easy identification for capture near the nest. If an ant during its recapitulation trajectory took longer than 5 min or went more than 1 m behind the feeder, the trial was recorded but terminated at that point. After the ant had completed both the sample and recapitulation trajectories, it was kept in a vial until the end of that morning's or afternoon's testing session. Thus, no ant was tested twice in the same morning or afternoon. Few, if any, ants would have been tested more than once from a release site. The six focal ants used in Fig. 2*C* were marked individually to allow recapture, but most other individuals were not marked.

The trajectories were digitized using GraphClick (Arizona Systems) and analyzed using MATLAB. To determine the distance-matched reference points (Fig. 5), each trajectory was divided into the half before the landmark ($y \leq 7$) and the half after the landmark ($y \geq 7$). The probe and reference points were then matched within the same half. Where more than one point on the sample trajectory shared the distance to the landmark of a probe point, then the point closest to the center of the route (where $y = 7$) was selected.

Evidence of Habitual Routes Past Landmark

A number of studies of desert ants in their natural environments (referred to in the main text) have shown that desert ants learn and follow habitual routes. These studies have shown that if an ant often travels between its nest and a particular food site, it tends to take the same routes, for instance, passing a particular side of a landmark on successive foraging trips and also if experimentally displaced back to an earlier point along its route. The routes often show small idiosyncracies between individuals. These previous studies have shown that the routes are controlled by visual memories of landmarks along the route, as well as by features around the goal. Building on the unambiguous and consistent findings from the previous studies, the current study explored issues of mechanism, rather than the existence, of route memories. As a consequence, during the main period of data collection, the recapitulation trajectories were recorded from release sites other than the feeder. Nevertheless, some additional data were collected that show the consistent individual routes past the landmark at this site. To keep the main text to a manageable length, these data have been reserved for Figs. S2–S4.

The additional evidence that the ants learn routes past the landmark comes from two sources. One source consists of pairs of trajectories collected in 2008 but during the training period before the main data collection commenced. Over this initial period, the reliability of the recapitulation trajectories increased. The second dataset was collected in 2006. That study used the same nest and landmark, with the feeder in approximately the same location, but with the landmark directly between the nest and feeder. It had two characteristics that made it better for showing that the ants were following routes, but at the same time worse for determining the navigational mechanisms at work. The first aspect is that ants traveled either side of the landmark, increasing the between-ant variation. The second aspect is that some homeward trajectories were recorded on a distant test ground that contained an identical landmark but provided a different panorama. As Narendra (1) found, route memories tend not to be well expressed when the panorama around landmarks does not match the habitual panorama. Nevertheless, short segments of route do appear to be expressed by some of the ants.

The first question that might be asked is whether, during its normal trajectories from the feeder, an ant follows a learned route past the landmark or whether the observed curves are simply the result of an automatic avoidance response to a large object (the landmark) that is superimposed onto a path integration vector home. That the homeward trajectories do reflect a learned response is suggested by the comparison between sample homeward trajectories on the training field versus on a test field recorded in 2006 (Fig. S2). If the paths round the landmark were simply produced by an innate avoidance behavior, then the trajectories guided by a path integration home-vector should be identical on training and test grounds. On the test ground, however, the landmark appears to have little influence on the shape of the homeward trajectories (Fig. S2*B*). The unfamiliar panorama at the test ground seems not to trigger the memories that guide the paths around the landmark.

The 2006 experiment was ideal for showing that ants follow the same routes on sample and recapitulation trajectories as the ants could travel either side of the landmark. Thus, in addition to any fine-scale differences in routes, there is also the obvious division into side. The sample and recapitulation trajectories that were recorded from the feeder tend to coincide at both levels (Fig. S3*A*). Recapitulation trajectories from the feeder location on a test ground with an identical landmark that was 200 m distant were generally less successful (Fig. S3*B*). The failure of most ants to follow their routes in this novel panorama agrees with the finding of Narendra (1) that the familiar accustomed panorama is required for reliable activation of the route memory. Nevertheless, it can be seen that there are some regions of striking coincidence. These segments where the route does appear to be recapitulated suggest that, where the route memory is activated, the encoding is indeed based on the landmark.

During the first days of training in 2008, ants replaced at the feeder for a recapitulation trajectory generally engaged only in search, or sometimes when they did run homeward again they interrupted their return, made a brief excursion, and then continued (Fig. S4*A*). By days 9–11, recapitulation trajectories from the feeder still sometimes showed brief excursions, but they were more often complete (Fig. S4*B*). Few recapitulation trajectories were collected over this initial period (all pairs from the feeder are presented here). The trajectories in the main text were all collected from day 11 onward, at which point no more recapitulation trajectories from the feeder were collected. The transition to reliable trajectories coincided with a marked improvement in the weather—from often cloudy and chilly to perfect blue skies from the afternoon of day 10 onward. It is not obvious why the early recapitulation trajectories were often unsuccessful. For the current analysis, it is important to note that those complete recapitulation trajectories generally coincide with their paired sample trajectories, supporting the contention that the ants are indeed learning routes past the landmark.

Differences in Behavior in Familiar Versus Unfamiliar Sector

The recapitulation trajectories were generally less well directed in the unfamiliar sector. The directness of a trajectory from a point was measured as the length of the path before leaving a disk of radius 3 m, divided by the shortest possible path length (i.e., the radius 3 m). Histograms comparing the trajectories from release points at (−2,0) and (−2,5,0) with those from (2,0) show that these normalized path lengths are greater from the release sites in the unfamiliar sector (Fig. S5*A*). A similar analysis was carried out at all probe points along trajectories originating at (−2,0) and (−2,5,0) in the unfamiliar sector to show where the transition between directed and undirected trajectories occurs (Fig. S5*B*). This transition is shown again in Fig. S5*C*, which defines long segments as trajectory length/radius >1.3. In the familiar sector, the path segments are mostly short, whereas in the unfamiliar sector, the path segments are mostly long.

1. Narendra A (2007) Homing strategies of the Australian desert ant *Melophorus bagoti*. II. Interaction of the path integrator with visual cue information. *J Exp Biol* 210: 1804–1812.

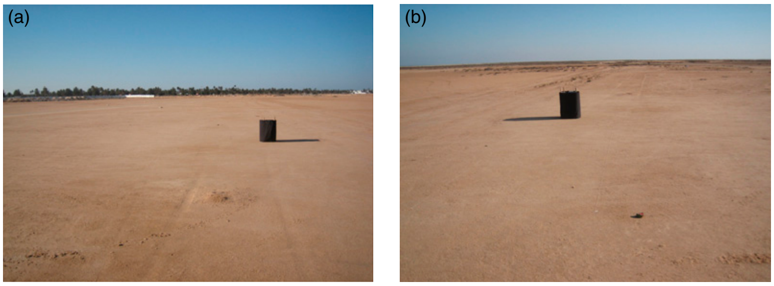

Fig. S1. Photographs of study site. (*A*) Viewed from nest (mound in foreground) to feeder, with landmark on right of route. (*B*) Viewed from feeder (watermelon in foreground) to nest, with landmark to left of route.

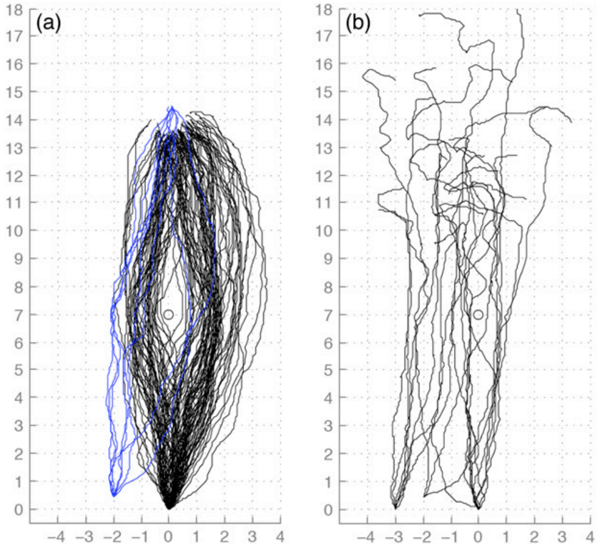

Fig. S2. Trajectories with home-vectors on training and test grounds. Trajectories collected in 2006 using same nest and landmark, but where the landmark is in line between the nest and feeder. (*A*) Initial homeward trajectories from feeder (black) and 2 m to left of feeder (blue). (*B*) Initial homeward trajectories of ants displaced from the feeder to a test site 100 m distant.

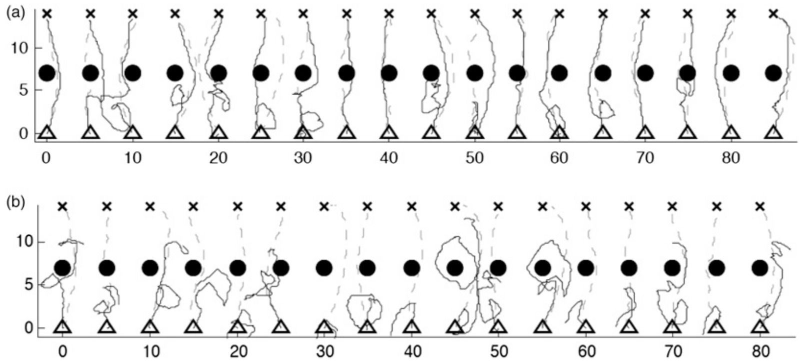

Fig. S3. Paired sample (dashed) and recapitulation (solid) trajectories from feeder. Trajectories collected in 2006. (*A*) Sample and recapitulation trajectories recorded on training ground. (*B*) Sample trajectories recorded on training ground. Recapitulation trajectories recorded on distant test field.

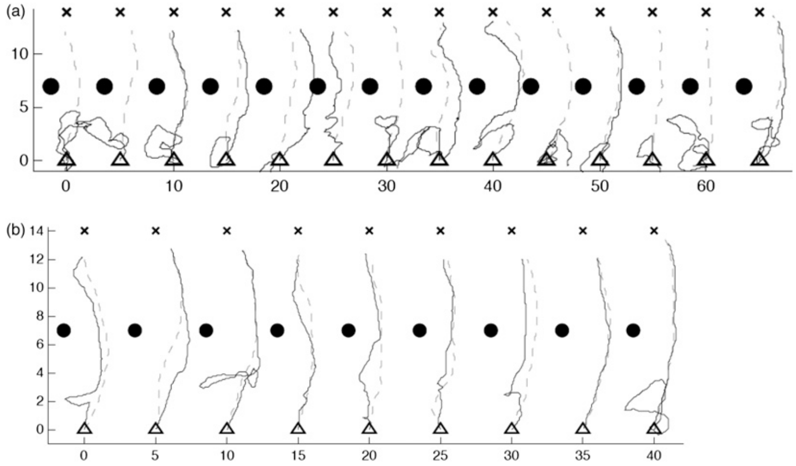

Fig. S4. Paired sample (dashed) and recapitulation (solid) trajectories from feeder. Trajectories collected in 2008 before data in main text were collected. (*A*) Paired trajectories on days 3–5. (*B*) Paired trajectories on days 9–11.

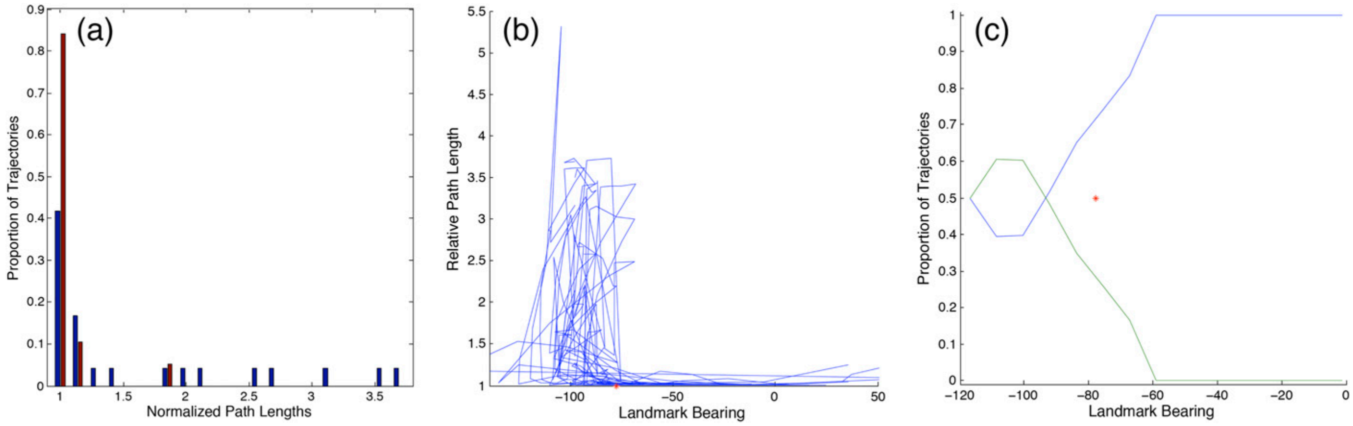

Fig. S5. Transitions in normalized path lengths between unfamiliar and familiar sectors. Path length to leave disk of radius 3 m divided by radius length. (*A*) Normalized path lengths from release sites at (2,0) in red and (−2,0) or (−2.5,0) in blue. (*B*) Normalized path lengths from probe points as function of landmark bearings at probe points. Trajectories released from (−2,0) or (−2.5,0). Red asterisk indicates beginning of familiar sector. (*C*) Proportion of long (>1.3, green) vs. short (<1.3, blue) normalized path lengths as a function of landmark bearings. Trajectories originating at (−2,0) or (−2.5,0). Red asterisk indicates boundary of familiar sector.

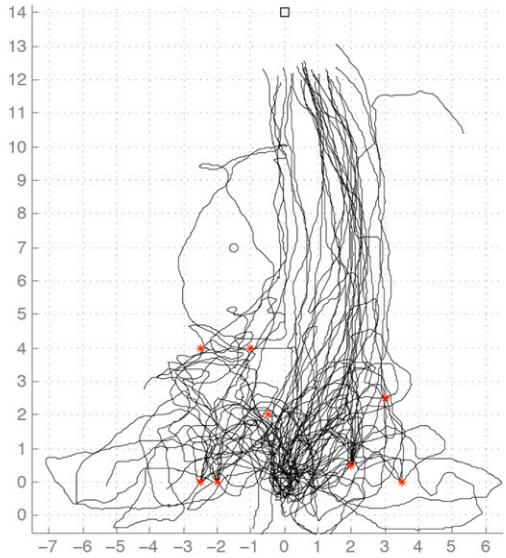

Fig. S6. Recapitulation trajectories that first went to feeder. Paths to feeder, which were not used in the analysis, are shown here, together with their continued paths (which were used in the analysis) after leaving the feeder.

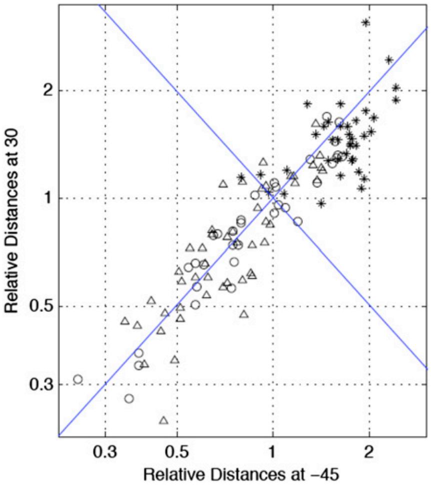

Fig. S7. The relationship between R-S distance ratios early in route segment (−45°) and late in the route segment (30°). Each pair of trajectories is illustrated by a single point. Trajectories in class 1 (○), class 2 (△), and class 3 (✱). Pairs with no change in R-S distance ratio would lie along main diagonal line. Pairs left or right of diagonal lines indicate that trajectories show convergence over segment. Points above or below diagonal lines indicate divergence. Most asterisks (26/31) lie in convergence areas.