Supplementary Methods

Initial training to the feeder was effected by moving a piece of watermelon incrementally, in steps of 1m, along the direct line from nest to feeder. Both landmark and feeder were removed overnight and replaced each morning.

Figure S1. (A) Photograph of the experimental area viewing the route from the side. The feeder (F) is to the left (at the foot of a bottle), the landmark (L) is in the center, and the nest (N) is inside the mound seen to the right.

The ants were captured using a technique that is standard for work with *C. fortis*. A circular plastic barrier, made from the top of a plastic bucket, is first placed to encircle the ant. The ant is then captured inside a small plastic tube. Because of the great agility of the ants, this process can often be quite lengthy. To release the ants, the plastic barrier is placed at the release site, and the tube is gently opened inside the barrier. Once the ant has emerged, and is seen to be still carrying its biscuit crumb, the barrier is removed.

Supplementary Results

Figure S2. Relationship between the locations of the start and end points of the intervals of confusion in the recapitulation trajectories. Locations are given as the distance from the feeder in the feeder-to-nest axis. Negative values indicate locations behind the feeder. Grey circles show the data for recapitulation trajectories from the feeder (see Figure 1C), black circles for recapitulation trajectories starting 2m past the feeder (see Figure 2A), and white circles for recapitulation trajectories starting 4m behind the feeder (see Figure 2C). Points below the line (Y=X) indicate where the net movement during the interval of confusion is backwards, away from the nest. This figure supplements Figure 1D in the main text.
Figure S3. (A) Initial trajectories used for recapitulation from feeder (N=18). The dashed lines indicate the positions used for the correlations in C-D. (B) Recapitulation trajectories from feeder. In contrast to Figure 1C in the main text, here the segments of trajectories judged to be following the route memories are highlighted, and the interval of confusion is in grey. (C-D) Correlations between the initial and recapitulation trajectories. The correlations used the x-coordinates where the trajectories (C) first reached y = 2 or (D) last reached y = 11. The solid points show ants which exhibited confusion (N=14), and the open circles show ants that recapitulated without confusion (N=4). The dashed line indicates where x-coordinates would be equal. The red lines are the least-squares best fits through all points. The shaded areas indicate where the initial and recapitulation trajectories are on different sides of the landmark. This figure supplements Figure 1 in the main text.
Figure S4. Histograms of the excess path lengths (EPL) of the trajectories shown in Figure 2A-D of the main text. As in the main text, the boxed inserts illustrate the manipulations schematically.

(A) (i) EPLs of extended homeward trajectories for which ants are displaced from the feeder to 18m behind the feeder. (ii) Schematic of the manipulation for (i). (iii) EPLs of recapitulation trajectories from 2m in front of feeder. (iv) Schematic of the manipulation for (iii).

(B) (i) EPLs of extended homeward trajectories for which ants are displaced from the feeder to start their trip 12m behind the feeder. (ii) Schematic of the manipulation for (i). (iii) EPLs of recapitulation trajectories from 4m behind the feeder.
Might the various levels of confusion be related to when the disruption to the ant caused by the capture and displacement occurs? When the ants are released for the recapitulation trajectories, the disruption occurs right at the beginning of the route. Moreover, they are released with a zero PI output vector, so that there is no guidance available from PI. In contrast, in the extended trajectories any potential disruption occurs long before the ant reaches its route, and it occurs when the ants have a 14m PI output vector directed towards the nest. With no further disruption, it is conceivable that in these trajectories the direction command from the PI output vector might continue to have some influence even after the PI state reaches zero.

The timing of the disruption was controlled for in a pair of manipulations for which ants were released with a 7m PI output vector, at a point 11m before the start of the route. As with the 18m extended trajectories (Figure 2D), the PI states would reach zero 4m before the ants passed the feeder and started the habitual route. Thus, the ants in both groups were released for the final (second) trajectory at the same point and with the same PI output vectors. These final trajectories confirm that the differences in confusion levels are not due to differences in guidance from PI, or to the timing of the disruption caused by the displacement process (Figure S5).

**Figure S5.** Extended homeward trajectories from -11m. (i) Histogram of the EPLs when the ants are first released to travel from –11m to –4m before again being caught and released from –11m. (ii) Schematic of the manipulation for (i). (iii) Histogram of the EPLs when the ants first travel 7m from the feeder to the landmark before being released at –11m to return all the way home. (iv) Schematic of the manipulation for (iii).
As on the first route, the recapitulation trajectories (Figure 3A) started off in the habitual directions (in this case for only 2.2±1.5m) and then interrupted the route-following with an interval of search (13.1±6.1m, 14/14 ants). Also as on the first route, the endpoints of the confusion (y=0.8±2m) were no further along the route than the start points (y=2.0±1.3m). An unexpected development during the course of this second experiment was that the amount of confusion during the recapitulation trajectories appeared to decrease. After the route had been in place 8 days, slightly fewer ants (11/13) showed a period of confusion, which started slightly later (after 2.7±1.2m) and lasted slightly shorter (8.7±7.5m) (Figure S2). The search started at y=2.4±1.1m and ended at y=2.5±1.9m from the feeder. After 11 days, the ants were generally recapitulating the route reliably. It is not obvious why the repetition effect should have persisted on the first route but have largely disappeared on the second route. Perhaps it was because the off-centre landmark allowed a more efficient or robust encoding of the route [11], or possibly because there was greater consistency in performance between days. It is also not obvious why the timecourse should be so slow. Once the repetition effect had mostly disappeared, the experimental focus was shifted to a study that showed that the route itself was encoded as a set of remembered heading directions. Of relevance to the current study, the previous analysis [11] suggested that the ants follow the remembered heading directions only when the landmark is experienced in the accustomed direction, and with approximately the accustomed retinal size.

Figure S6. Decrease in levels of confusion during the recapitulation trajectories on the asymmetric route.
(A(i)) Recapitulation trajectories during days 3-5. Same data as Figure 3B in the main text. (ii) Histograms of excess path length of the recapitulation trajectories (i).
(B(i)) Recapitulation trajectories during days 9-11. (corresponding to Figure S3B). (ii) Histograms of excess path length of the recapitulation trajectories (i).
Figure S7. Recapitulation trajectories on the asymmetric route (Corresponding to Figure 3, Main text). The recapitulation trajectories from which the data in Figure 3C-D was calculated. 

(A(i)) Recapitulation trajectories after the initial trajectories that passed to the left of the landmark (corresponding to Figure 3C(i)). (ii) Recapitulation trajectories after the initial trajectories that passed to the right of the landmark (corresponding to Figure 3C(ii)).

(B(i)) Recapitulation trajectories after the initial trajectories that passed to the right of the habitual route (corresponding to Figure 3D(i)). (ii) Recapitulation trajectories after initial trajectories along the habitual route (corresponding to Figure 3D(ii)).