

Report

A Common Frame of Reference for Learned and Communicated Vectors in Honeybee Navigation

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Summary

Humans draw maps when communicating about places or verbally describe routes between locations. Honeybees communicate places by encoding distance and direction in their waggle dances [1]. Controversy exists not only about the structure of spatial memory but also about the efficiency of dance communication [2–5]. Some of these uncertainties were resolved by studies in which recruits' flights were monitored using harmonic radar [6, 7]. We asked whether the two sources of vector information—the previously learned flight vector to a food source and the communicated vector—are represented in a common frame of spatial reference. We found that recruits redirect their outbound flights and perform novel shortcut flights between the communicated and learned locations in both directions. Guidance by beacons at the respective locations or by the panorama of the horizon was excluded. These findings indicate a spatial reference based on either large-scale vector integration or a common geocentric map-like spatial memory. Both models predict a memory structure that stores the spatial layout in such a way that decisions are made according to estimated distances and directions. The models differ with respect to the role of landmarks and the time of learning of spatial relations.

Results and Discussion

Before our honeybees were recruited by the waggle dance, they had performed their exploratory orientation flights and visited other food sources [1, 8, 9]. We asked whether the stored vector information from previous foraging flights is somehow compared to the information received during dance communication. Recruits may be reminded of their own experience and be motivated to visit their food source [5]. They may choose between the previously learned and the communicated vector information, depending on the difference in location. Recruits may also first fly either to the previously experienced food source or according to the communicated vector but may then correct themselves and follow a novel shortcut to

the other respective location. In earlier studies, it was not possible to test all of these possibilities because doing so requires complete flight tracking, monitoring the communication process within the hive on an individual basis, and a protocol of the recruits' former foraging experience. In the experiments reported here, all of these requirements were met.

We shall first deal with the outbound flight of recruits tested in the main experiment, in which the dance-indicated feeder (FD) and the trained feeder (FT) were positioned 650 m from the hive (H) and the angle between the directions H > FD and H > FT was either 30° or 60° during tests (Figure 1). Recruits that did not leave the hive had followed on average only 6.8 dance rounds (n = 41). Animals that followed on average 20.0 dance rounds terminated their outbound journey within the first 200 m and returned to the hive before reaching either FD or FT (n = 18). We found no statistical difference (analysis of variance [ANOVA]) between these animals with interrupted flights heading off toward FD or FT in both the 60° or 30° groups. In the case of complete flights (n = 55), the average number of dance rounds followed in the 60° and 30° groups was not significantly different (ANOVA). Recruits arrived at FD after following 23.2 ± 10.4 (60° group) and 20.7 ± 11.0 (30° group) dance rounds and at FT after following 8.4 ± 3.8 (60° group) and 16.4 ± 4.5 (30° group) dance rounds. Recruits flying toward FD in both the 30° and 60° groups followed significantly more dance rounds than those flying toward FT (p < 0.001, ANOVA).

These results indicate that dance communication involves two components, a motivational and an instructive component. In the first case, bees fly according to their foraging memory; in the second case, according to the communicated information. The latter requires more information transfer. The motivational component appears to activate the memory of former foraging experience, possibly including a reminder component transferred by trophallaxis, because the dancing bee foraged on the same kind of pure sucrose solution as the recruits had days before. A goal-specific odor known as a reminder stimulus [5] was not involved in our experiments. The straight flights toward FD over a distance of 650 m without any odor as a potentially guiding cue corroborate earlier findings about the efficiency of the instructive component [6].

Figures 2A–2C show the complete outbound flights up to a radius of 600 m. Our analysis of the directedness of the outbound flights focused on the radius of 250 m around the hive because recruits applied additional strategies beyond this distance in the 30° test situation (see below). We found two distributions in the 60° tests (Figure 2A), one directed toward FD (n = 6) and one directed toward FT (n = 6), and three distributions in the 30° test (Figure 2B), directed toward FD (n = 7), toward FT (n = 12), and along a midline between these two directions (n = 4). These distributions were significantly different from each other (see Figure 2 legend and the Supplemental Experimental Procedures available online). The midline flights in the 30° tests indicate a different flight strategy possibly resulting from some form of vector integration of the two sources of information, dance-transmitted direction and learned direction. Beyond a distance of 600 m, three animals flew toward FT, and one flew toward FD.

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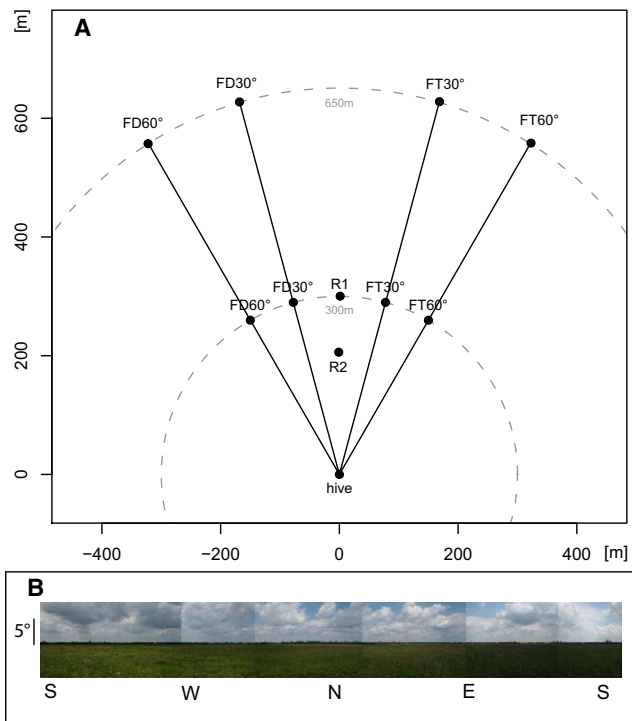


Figure 1. Training and Testing Layout

(A) The experiments were carried out on a flat, open, horizontal pasture (see Figure S1) with no rising landmarks in the vicinity of 500 m (as seen from the training locations of recruits and dancers). FD and FT indicate the locations of the dance-indicated and the trained feeders, respectively. 30° and 60° refer to the respective tests in which the angular separation between these two locations as seen from the hive was either 30° or 60°. The main experiment was performed for average distances of 650 m (schematically indicated by the outer dashed circle), the control experiment with a distance of 300 m (inner dashed circle). The location of FD and FT varied in subsequent experiments (see Figure S1), but the flight traces in Figure 2, Figure 3, Figure 4, and Figure S2 were normalized such that FD lies to the left and FT to the right. The following normalization procedure was applied: the angular deviation of each radar point in any particular experiment was calculated relative to the connection between the hive and the particular location chosen by the animal for its destination. These relative angular deviations were used to relate the respective radar points to the normalized direction. For example, if an animal flew from the hive toward F8 (see Figure S1), which in this particular 30° experiment was the location indicated by the dance (FD), the angular deviation of each radar point to the direct connection between hive and F8 was calculated and used to reconstruct the corresponding normalized radar points along the connection between hive and FD30°.

(B) Panorama of the horizon as seen from a viewpoint halfway between FT and FD. The angular fluctuation was below 2° within a radius of 500 m around this viewpoint. A visual angle of 2° is considered to define the angular resolution of the bee eye [21, 22]. It is therefore concluded that bees did not approach one of the test sites by stepwise matching of the memory of the panorama as seen at one of these sites with that seen at a particular location but rather used local ground structures embedded in the sun compass when localizing themselves within the test area [7].

Because such midline flights were not found in the 300 m control experiments (see below), it is likely that vector integration involves a distance component. More animals flew toward FT than toward FD in the 30° test situation (FD, 6 flights; FT, 15 flights; 2 midline flights did not end up in the close vicinity of FD or FT), a trend that was also seen in the control experiments at the 300 m, 30° test situation (FD, 5; FT, 11; see below).

Two additional characteristics appeared in the flights of the 30° group. (1) Animals might head off toward FD first but then

correct their flights and steer toward FT (redirected flights; see also Movie S1F). Such redirection occurred only in initially FD-directed flights, and only in the 30° test situation. In the 650 m training and test groups, 3 out of 6 initially FD-directed flights were redirected toward FT (#35, 53, and 54; Figure 2B). In the control experiment (see below), animals were trained and tested at distances of 300 m. Only 1 of 5 redirected flight was observed, and this flight was also initially directed toward FD and occurred in the 30° group (#81; Figure 4B). (2) Animals in the 30° test group might not return to the hive directly after arriving at FD or FT but might return via the other location (see Movies S1H–S1P). We call these novel shortcut flights “cross flights” and applied two definitions, a strict definition in which recruits needed to begin these flights within a 50 m radius around FD or FT and arrive at the other location within a 50 m radius (Figure 3), and a broader definition in which a clear direction toward the other location could be detected even if the flight was not initiated within a 50 m radius around FD or FT (Figure S2). The 50 m radius was chosen because we found that two-thirds of the last sharp turns before departure from FD or FT occurred within 50 m around these locations. Furthermore, the cross points of the departure flights from FD or FT with the 50 m radius indicated that no animal left the location through the 180° sector opposite to the other location. Five of 23 flights were performed according to the strict definition (3 from FT to FD and 2 from FD to FT; Figure 3), and 6 out of 26 flights according to the broader definition (Figure S2). Cross flights of the strict definition were performed after following on average 20.7 ± 11.9 dance rounds. These values lie between the respective ones for FD (20.7 ± 11.0) and FT (16.4 ± 4.5) flights without cross flight, and the differences are not significant. Note that recruits in the main experiment performed no cross flights if the distance between FD and FT was the same as the distance between FD or FT and the hive. A few animals in the main experiment performed flights that resembled portions of a cross flight (e.g., animal #29 in Movie S1G). Cross flights according to the strict definition were also performed in the control experiment (see below), 7 of 16 flights in the 30° test, and 3 of 15 flights in the 60° tests (Figure 4).

Cross flights are novel shortcut flights between two locations, FD and FT. These flights could not have been guided by the structure of the panorama or beacons at these sites. FT was an empty place during the tests; FD was marked by a person sitting down on the grass and who may have been visible within a radius of 30 m (visual catchment area, Figure 3; see also Figure 1 legend). We therefore conclude that the common reference for experienced and dance-communicated locations allows for novel shortcut flights between two locations that can be approached only by means of their spatial relations.

We next asked whether the spatial relation is defined by the metric distance or the angular difference. A control experiment was performed for distances of 300 m between H and FD and between H and FT (Figures 4A–4E). The metric distance between FD and FT for the 60° arrangement equaled that of the 30° arrangement of the main experiment. Cross flights occurred again in the 30° arrangement, but also in the 60° arrangement, indicating that the metric distance was the main parameter. The occurrence of cross flights in the 300 m, 60° test situation indicates that at such shorter distances, recruits crossed over to the respective other location even if their distance equaled that back to the hive.

If recruits would steer toward FD and FT as locations rather than as endpoints of vectors, they might also do so from

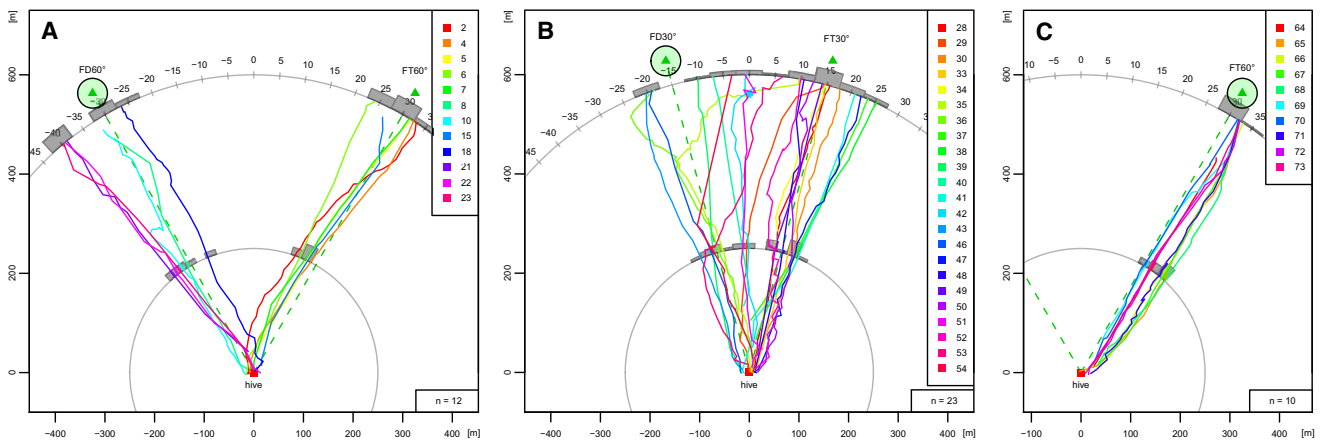


Figure 2. Outbound Flights during Test Conditions and during Training

- (A) 60° test group.
- (B) 30° test group.
- (C) During training.

The distributions at 250 and 600 m are given in bar histograms (bin width 5°). The circle around FD (radius 30 m) marks the estimated visual catchment area at FD. Some of the flights ended before reaching the 600 m circle because bees flew low and escaped detection by the radar but were later picked up again when crossing the 600 m line, indicating a straight flight toward the respective site. Note that four bees of the 30° test group (B) initially flew along the midline between the directions toward FD and FT. One arrived close to FD, and three arrived close to FT. One arrived close to FD, and three arrived close to FT. Two bees departed in the direction of FD but then redirected their flights toward FT, one about halfway and one when already close to FD.

Statistics of directedness: the distributions were compared at a radius of 250 m around the hive. The distance of 250 m was chosen because this was the furthest distance before redirected flights started.

(A) FD-directed flights: mean vector (μ) = $-33.01^\circ \pm 5.8^\circ$ (standard error of the mean = 3.2°); FT-directed flights: mean vector (μ) = $24.3^\circ \pm 2.7^\circ$ (standard error of the mean = 1.5°).

(B) FD-directed flights: mean vector (μ) = $-16.7^\circ \pm 3.6^\circ$ (standard error of the mean = 1.7°); FT-directed flights: mean vector (μ) = $17.9^\circ \pm 4.4^\circ$ (standard error of the mean = 1.5°); midline-directed flights: mean vector (μ) = $1.5^\circ \pm 1.1^\circ$ (standard error of the mean = 0.9°).

Watson's F test for two circular means gives statistically significant differences ($p < 0.001$) for all pairwise comparisons between flights directed toward FD, toward FT, and along a midline. The Kruskal-Wallis test for angular distribution gives statistically significant difference ($df = 2$, $p(\chi^2) < 0.05$) for the comparison of all three groups. (See Supplemental Experimental Procedures.)

a position other than the hive. We therefore performed additional control experiments in which we transported recruits from the hive entrance to a release site, R1 in the 650 m experiment (300 m from the hive) and R2 in the 300 m experiment (200 m from the hive; see Figure 1A). In both cases, the release sites were positioned at half of the angle between H and FD and between H and FT. Six of the 10 recruits tested flew straight back to the hive; the others first performed vector flights according to either $H > FD$ or $H > FT$ directions and then returned back to the hive along straight flights. This behavior resembles earlier findings [7]. Foragers predominantly returned to the hive along straight flights after applying their working memory (either the vector $H > FD$ or $H > FT$). These straight flights represent novel shortcut flights back to

the hive. In earlier studies, well-trained foragers flew also to the feeding site [7], but the low salience of FT in the experiments reported in the present study may have led to the dominance of hive-returning flights.

The return flights to the hive in the main experiment often deviated from the 180°-rotated outbound flight (Figure S3). Flights along the midline were most frequent in the 650 m, 30° test situation. Bees performing partial cross flights were more likely to return to the hive along the midline, indicating shortcutting behavior.

Taken together, our findings document a surprising complexity and richness of novel behaviors in honeybee navigation and communication. Information transmitted during the waggle dance led to navigation according to the encoded

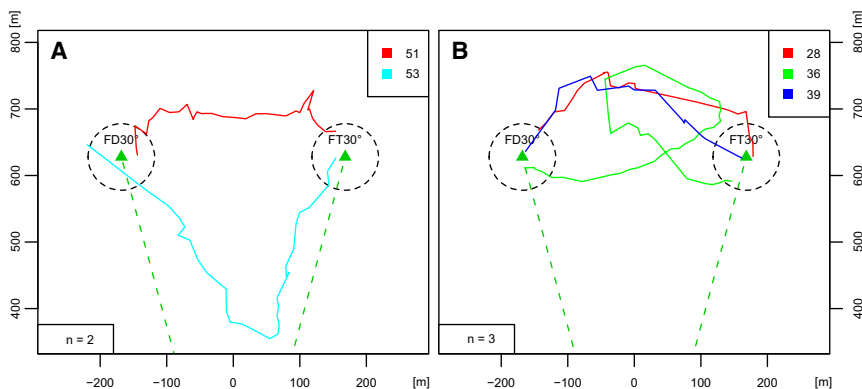


Figure 3. Cross Flights of Recruits

Here we apply the strict definition (arrival at both locations within a radius of 50 m; see text) and show the cross flights occurring in the 650 m, 30° experiment. The circles around FD and FT (radius 50 m) mark the areas around the respective sites which the animals had to reach during their cross flights.

(A) Two animals (#51 and #53) arrived at FD and flew to FT.

(B) Three animals arrived at FT and flew to FD.

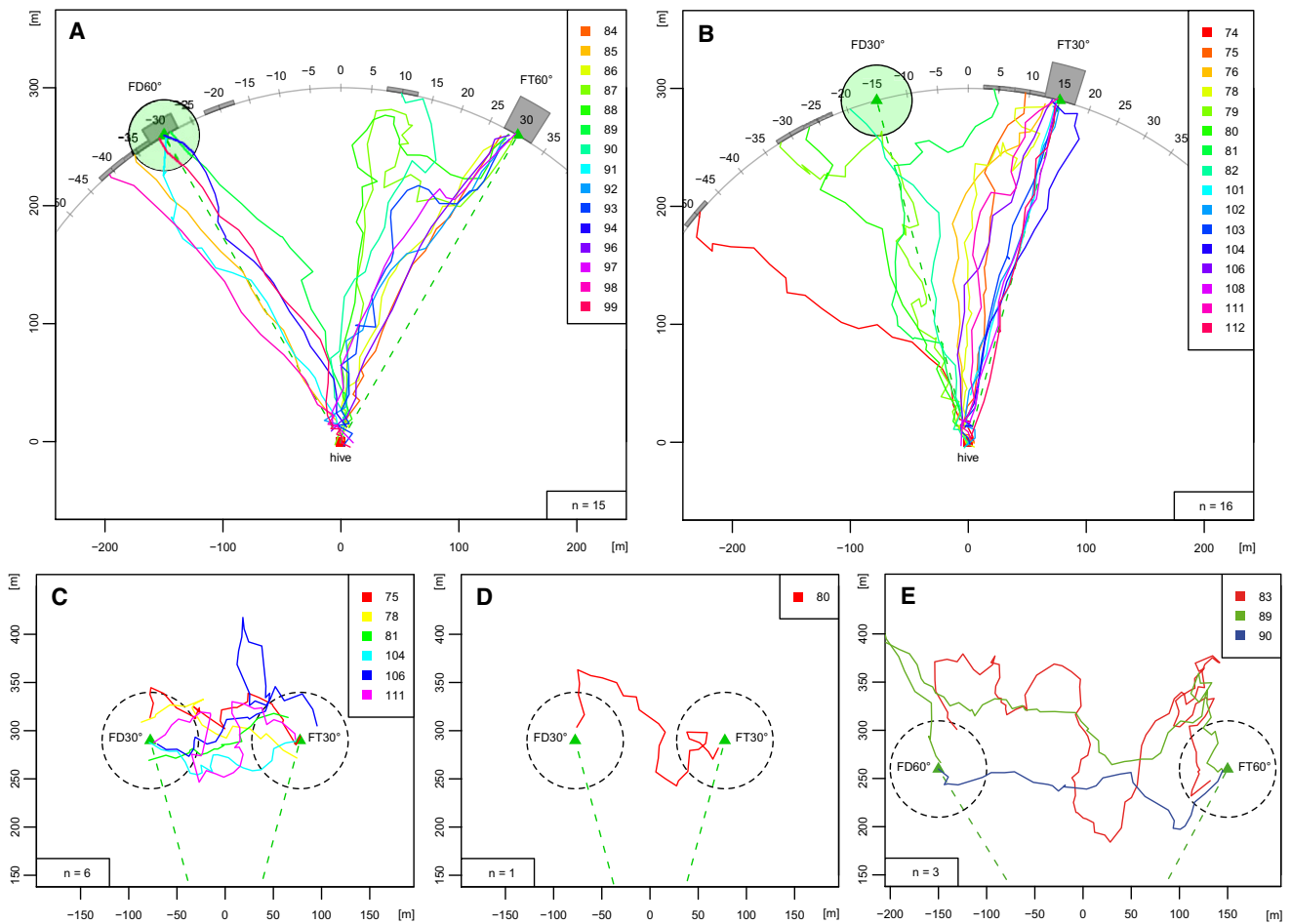


Figure 4. Outbound Flights and Cross Flights in the 300 m Control Experiment

(A) Outbound flights in the 60° experiment.

(B) Outbound flights in the 30° experiment.

(C and D) Cross flights in the 30° experiment after arriving at FT (C) and after arriving at FD (D). The dashed circles around FD and FT (radius 50 m) define the areas the animals had to reach for the strict definition of cross flight.

(E) Cross flights in the 60° experiment.

Statistics of directedness: the distributions were compared at a radius around the hive of 250 m.

(A) FD-directed flights: mean vector (μ) = $-21.3^\circ \pm 9.6^\circ$ (standard error of the mean = 4.6°); FT-directed flights: mean vector (μ) = $12.9^\circ \pm 6.1^\circ$ (standard error of the mean = 2.4°).

(B) FD-directed flights: mean vector (μ): $-18.7^\circ \pm 12.4^\circ$ (standard error of the mean = 6.0°); FT-directed flights: mean vector (μ) = $9.7^\circ \pm 4.0^\circ$ (standard error of the mean = 1.6°).

spatial information at a high degree of probability and precision. Odors emanating from the dance-indicated location were not required for successful arrival at this location as proposed in the past [10] and were not necessary for motivating recruits to follow the spatial instructions of the dance [5]. Furthermore, experienced and communicated spatial information were integrated into a common navigational reference. Dance attendance might motivate a recruit to depart for its learned site (FT) when it followed a lower number of waggle runs. After arriving at FD or FT, recruits either returned home or crossed over to the other site. Cross flights were independent of the actual location of FD and FT and could not depend on any foraging flights to natural food sources (see [Experimental Procedures](#)). Cross flights depended on the absolute distance between the two locations. Thus, spatial information from both the dance and former foraging experience needs to be processed in a common reference. Novel shortcut behavior also occurred during return flights to the hive both under

normal test conditions when animals departed from the hive and after they were released at an unexpected site.

Two rather closely related models may explain these data: an extended vector integration model or a cognitive map model. In the first case, dance attendance activates a common working memory that stores the vector toward FT via a motivational reminder component and the vector toward FD via the instructive component. Redirected flights, cross flights, and shortcut homing flights are thought to result from large-scale vector integration. Path integration has been conceptualized as a process of continuous updating of path segments in an accumulator providing the animal with the information for direct returns [11]. Our results, however, indicate that bees store a vector from former experience or from dance communication in such a way that it can be computed with another vector that the animal had just applied. This procedure resembles on a formal basis the computation of triangulation in trigonometry, and thus a procedure to capture spatial relations.

Furthermore, because bees appeared to weigh the alternatives of flying back to the hive or to the other destination (either FD or FT) depending on the respective distances, we conclude that decisions were also made on the basis of estimated distance to the intended goal. The bees performed cross flights when the distance between FD and FT was shorter than that to the hive (or at an equal distance, if the absolute distances between hive and FD or FT were much shorter, as in the control experiment). Triangulation (or integration of remembered vectors) may thus involve estimates of distances to the two other locations. We do not know how complex the bees' vector-based system may be, but we also cannot exclude the possibility that the reference used in navigation may indeed reach beyond a single triangle connecting three points. At least we can exclude the possibility that the processes involved in computing information transmitted in dance communication involve nothing other than the application or discounting of that information.

The cognitive map model assumes that landmarks in the environment are stored by multiple spatial relations in such a way that animals recognize landmarks and use them for localizing themselves and aiming toward a goal [12, 13]. Bees derive the sun compass-related directions of flights and the corresponding dance directions from landmarks when the celestial cues are not available [1, 14]. They learn the sequence of landmarks during foraging flights along a route and even adjust their odometer to that sequence [15]. Thus, flight vectors are not represented in their navigation memory without reference to landmarks embedding the triangulated space to landmarks. In the past, it has been difficult to support a map-like navigation memory because guidance by beacons at the respective goals and stepwise matching procedures with reference to the panorama were not excluded [16–19]. Neither kind of cue was available to the bees in our experiments. Redirected and cross flights as well as homing flights are indeed novel shortcuts and meet the strict requirements for a cognitive map in Tolman's original sense [12]. Thus, the map model assumes a memory that cannot be established during route flights but requires learning of landmark relations during multiple exploratory flights as are performed by young bees during their first excursions from the hive [1, 20].

The two models differ in two respects: the role of landmarks (spatial relations along a route or global relations) and the timing of learning (during foraging or during exploration). Further experiments are needed to examine the relations of these navigation memories. In any case, memory as established during large-scale vector integration and memory established as map memory differ only in the richness of knowledge about spatial relations between landmarks. It is well possible that both forms of memory exist side by side, and indeed it has been concluded from radar tracking experiments that vector-based memories dominate navigation behavior in bees when they find themselves in a conflict [7]. The basic cognitive components in navigation and communication in bees and humans may not be so different.

Experimental Procedures

We trained a group of honeybees to a feeder (FT). We called these bees recruits because they would later be recruited by dancing bees. Dancing bees were trained to another feeder (FD). Both FT and FD were switched in subsequent experiments with the aim of avoiding landscape features that could serve as potential guiding posts to either of the two locations and avoiding any bias toward one of the places possibly resulting from former experience with natural food sources (see also below). Four

conditions of FT and FD locations were tested (Figure 1A; see also Supplemental Experimental Procedures). The distance between the hive and FT or FD was on average 650 m in the main experiment and 300 m in the control experiment. In both cases, the angular difference between $\text{hive} > \text{FT}$ and $\text{hive} > \text{FD}$ directions was either 30° or 60° . When the dancers danced, recruits had experienced during the previous day that their own food source (FT) did not supply food any more. Recruits performed several flights in which they experienced the closed feeder. Some of these flights were monitored with the harmonic radar, and in no case did the bees explore beyond the immediate surroundings of FT. When the two bees trained to FD danced, recruits attended these dances and their behavior was recorded on video. We knew that FD was a location the recruits had not foraged at before because of the lack of natural food supply; we also kept a complete protocol of all bees visiting FD. When a recruit departed from the hive after following a dance, its flight path was radar tracked. A total of 124 flights were recorded. There was no food source or any cues at FT during these test flights, and a person was sitting low on the ground at FD training the two bees that performed the dances. Guidance by beacons close to the two locations or the panorama of the horizon was excluded (Figure 1B), and bees navigated via the ground structure. The experiments were carried out in late summer and autumn when very few natural food sources were available after the grass had been cut.

Supplemental Information

Supplemental Information includes three figures, one table, Supplemental Experimental Procedures, and one movie and can be found with this article online at [doi:10.1016/j.cub.2011.02.039](https://doi.org/10.1016/j.cub.2011.02.039).

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