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Title

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Journal

Insect Science, 27(2)

ISSN

1672-9609

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Publication Date

2020-04-01

DOI

10.1111/1744-7917.12651

Peer reviewed

Author running head: *B. kheradmand et al.*

Title running head: *Landmarks influence foraging and waggle dance*

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ORIGINAL ARTICLE

Influence of visual targets and landmarks on honey bee foraging and waggle dancing

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Abstract

Animals use diverse sensory stimuli to navigate their environment and to recognize rewarding food sources. Honey bees use visual attributes of the targeted food source, such as its color, shape, size, direction and distance from the hive, and the landmarks around it to navigate during foraging. They transmit the location information of the food source to other bees if the food source is highly rewarding. To investigate the relative importance of these attributes, we trained bees to feeders in two different experiments. In the first experiment, we asked whether bees prefer to land on (a) a similar feeder at a different distance on the same heading or on (b) a visually distinct feeder located at the exact same location. We found that, within a short foraging range, bees relied heavily on the color and the shape of the food source and to a lesser extent on its distance from the hive. In the second experiment, we asked if moving the main landmark or the feeder (visual target) influenced

This is an Accepted Article that has been peer-reviewed and approved for publication in the Insect Science but has yet to undergo copy-editing and proof correction. Please cite this article as [doi: 10.1111/1744-7917.12651](https://doi.org/10.1111/1744-7917.12651).

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recruitment dancing for the feeder. We found that foragers took longer to land and danced fewer circuits when the location of the food source or a major landmark associated with it changed. These results demonstrate that prominent visual attributes of food sources and landmarks are evidently more reliable than distance information and foraging bees heavily utilize these visual cues at the later stages of their journey.

Key words *Apis mellifera*; navigation; learning; foraging; visual orientation

Introduction

Animals depend upon learning environmental cues to exploit resources. They use multiple modalities and learn multiple stimuli, including visual, olfactory, tactile, and acoustic attributes of the resource or its immediate surrounding (Bingman & Cheng, 2005; Gould & Gould, 2012). This learning is particularly important for central place foragers, animals that navigate from their nest to target resources and back (Dyer & Gould, 1983; Wehner *et al.*, 1996; Able, 2001). To achieve this complex task, animals use multiple navigation strategies. Birds, mammals, and insects such as honey bees can path integrate by calculating the overall direction and distance between a starting point and a destination by memorizing the directions and distances of path segments (Mittelstaedt & Mittelstaedt, 1982; Muller & Wehner, 1988; Chittka *et al.*, 1995; Etienne *et al.*, 1996; Stone *et al.*, 2017). Such navigation can rely on visual cues, such as the position of moving celestial objects, and visual attributes of the resource and fixed, conspicuous objects nearby (Greene & Cook, 1997; Collett & Graham, 2004).

Honey bees can measure direction from solar information (Rossel & Wehner, 1984) and distance, mainly from integrating the amount of visual motion, optic flow, passing by their eyes (Esch & Burns, 1995; Srinivasan *et al.*, 1997; Dacke & Srinivasan, 2008). A few animals, such as humans

and perhaps even honey bees (Menzel & Greggers, 2015, but see Cruse & Wehner, 2011), form a cognitive map of the environment and assess their location on this map-like representation. A very common strategy is to also use landmarks, objects that are easily recognizable, conspicuous, spatially persistent, and in the proximity of target. The size, color, shape, and patterns of these landmarks, of objects *en route*, and the resource itself can all be used (Cartwright & Collett, 1982; Dyer, 1996).

Foraging bees are excellent models of animal navigation because they make multiple return trips per day to good resources (von Frisch, 1967). Bees develop diverse memories from multiple modalities, and it is known that some memories are preferred over others due to a hierarchy of relevance and reliability that allows bees to use available information to forage efficiently (Gould & Towne, 1988). We investigated the relative importance of two of these memories: distance information and visual attributes of the food source.

We hypothesize that bees rely on the memories of features that are associated with a food source, such as its exact shape and color, rather than memories of features that are different for different individual resources of the same type, such as their distance or direction. We only tested the effect of distance compared to visual features of the target in our experiments. Researchers train bees to a specific location by gradually moving the feeder away from hive entrance. This demonstrates that once bees learn the visual features of a feeder, they can follow it around as it moves as long as the moving speed is slow and the food source is conspicuous (von Frisch, 1967). Many studies have shown the dominance of landmarks in finding food sources, and the target itself is one of the most important visual cues used in honey bee navigation (von Frisch, 1967; Cartwright & Collett, 1982; Chittka & Geiger, 1995).

Honey bees are also a useful model for studying navigation because of their ability to communicate the location and the quality of a food source to nestmates via the waggle dance (von Frisch, 1967). Bees forage optimally and therefore minimize foraging trip duration and flower manipulation time while advertising the resources that have the highest returns to other bees

(Waddington & Holden, 1979; Wells & Wells, 1983; Pyke, 1984). For highly profitable resources, bees increase the number of dance circuits that they perform and visit the food source more frequently (von Frisch, 1967). This perceived profitability depends on several factors, such as the hunger state of the hive (which is contingent upon past and present resource availability), current resource quality, colony need for the resource (Seeley, 1992), overall hive activity levels, and even the weather and plant species being visited (von Frisch, 1967). Thus, the repetition of waggle dance circuits by an individual dancer integrates multiple sources of information and is a readout of individual forager judgments while also reflecting colony need and food availability.

The ephemeral nature of floral resources requires individual foragers and the colony to constantly seek out new food sources. Bees are known to dance for newly-discovered food sources that maintain profitability over the course of a few visits (von Frisch, 1967), but we asked if a new food source might be considered more valuable by a forager that has repeatedly visited the same food source for an extended time. In other words, will novel food increase dance effort? Because bees consider multiple properties of a nectar resource such as its flow rate, sugar concentration, and distance from the hive in determining food quality (Dyer, 2002), we wished to test a manipulation that would not alter the net energy that bees could obtain from the resource significantly. We therefore changed the landmarks surrounding the food to test if this would alter dancing. Specifically, we tested if associating a relatively inconspicuous food source with a nearby conspicuous landmark would result in bees relying on the landmark and if separating the food source from the landmark by a very short distance would change forager visits and dance effort.

We therefore conducted two experiments. The first tested the relative importance of visual properties of the target and its perceived distance from the hive on forager bees' landing choice. Given the importance of visual attributes of the target established by the first experiment, in our second experiment we explored whether altering a major landmark associated with a food source would alter foragers landing behavior and their perceptions of food value, as measured by the number

of dance circuits performed by each forager upon returning to the nest and the number of visits per unit time to the food source.

Materials and methods

Experiment 1: Visual properties of a target versus its distance

We performed these experiments in June–October 2016. We used 10 colonies of *Apis mellifera ligustica* located in an apiary at UCSD Biological Field Station in La Jolla, California. We trained bees to a feeder in a tunnel that amplified the optic flow they perceived. On each day, we began training by putting the feeder close a focal hive. We used a new focal hive each day, but it was possible that bees from other hives could visit the feeder and become trained to the tunnel. However, this should not have influenced our results since our goal was to ensure that we used bees from multiple colonies to ensure adequate colony-level replication.

Tunnel

Our tunnel was 9.5 cm wide, 18 cm tall, and 8 m deep. At its 8 m end, the tunnel was blocked with white cardboard. The tunnel rested on 80 cm tall sawhorses and was positioned in an open field such that no overhead landmarks were visible to bees inside the tunnel. Tunnel walls were lined with alternating 2.5 cm black and white vertical stripes on the sides. The tunnel floor also had the similar stripes perpendicular to its long axis (Fig. 1A). The tunnels were covered by a thin black mesh on the top that allowed bees to see the sky, UV light, and polarized light patterns, but forced bees to fly through the tunnel to access the feeder (Fig. 1). Before each test, tunnel walls and the mesh were cleaned with water to remove odor marks. Although this cleaning method may not have removed all odors marks, the tunnels were built in 1.2 m long segments that were frequently and randomly shuffled during the training phase to avoid odor mark accumulation that could guide bees to the

feeders. Most importantly, prior to testing of each bee, the wall segments were cleaned and shuffled again.

Artificial flowers (feeders)

We used two types of feeders distinguished by their distinct colors and shapes (Fig. 1 C, D). The first type was a bright yellow cuboid (3.9 cm × 3.9 cm × 1.8 cm) with a cylindrical hole in the center (1 cm wide, 0.8 cm deep) holding the sucrose (hereafter referred to as YS, short for Yellow Square). The second type was bright blue circular Petri dish (diameter 5.2 cm, height 1.4 cm) painted blue on the outer side and bottom and was covered with an aluminum mesh (hereafter referred to as BR, short for Blue Round). These feeders were filled with 2.0 M unscented sucrose during the training phase. During the testing phase, we used empty unscented feeders that were placed at one or more of three possible distances in the tunnel: 2.4 m, 4.8 m, and 7.2 m. Based upon the ease with which bees oriented to and landed on these feeders, they were evidently easily noticed by bees inside the tunnels, and served as targets to which bees would navigate.

Training

Following standard methods (von Frisch, 1967), bees were trained to a feeder placed on top of a flat white disc (25 cm radius) on a tripod (80 cm high). The tripod was initially set up at the entrance of a focal hive and gradually moved to the tunnel entrance. We then placed the feeder inside the tunnel, resting on a transparent plastic stand (4 cm high, as shown in Fig. 1C, D) and slowly trained the bees to fly 4.8 m inside the tunnel. Bees then visited the feeder for at least 2 h until they were proficient in flying through the tunnel to reach the feeder. At the beginning of this 2 h period, bees were individually marked on their thoraces with different colors of acrylic paint. On average, each bee visited the feeders every 7-10 min, which resulted in 12-15 total visits at the feeder's final location per

bee before testing. In all conditions except one, the training feeder was YS, and in one condition (designed to test for innate color preference), the training feeder was BR (Table 1).

Testing conditions

In each testing condition, artificial flowers of certain types were put at different distances in the tunnel (Table 1). Based on the options, bees landed at the feeder at the distance to which they had been trained (4.8 m), the closer feeder (2.4 m), the more distant feeder (7.2 m) or did not land at all during 2 min (see below). To begin the testing phase, we allowed one bee to enter the tunnel and then blocked the tunnel with a piece of white cardboard, thereby limiting our assay to one bee at a time and preventing the choices of other bees from potentially influencing the behavior of the focal bee. A choice was defined as a bee landing on a specific feeder or not landing on any feeder during the time it spent inside the tunnel. The landing choice, flight time until landing, and behavior prior to landing was recorded by two observers. Observers moved frequently along the length of the tunnel to avoid becoming landmarks. Each condition was tested on 20 different bees, except for condition 0, which was tested on 29 bees. In total, we tested 189 bees (approximately 17 bees per colony). Based on previous observations, we knew that bees had a wide range of visit rates. During 2 hours, a few bees visited the feeder inside the tunnel more than 20 times, while some had fewer than 10 visits, and most bees returned around 15-18 times. By randomizing the testing condition for each bee, and testing a large number of bees in each condition over multiple days, we ensured that differences between bees in different conditions were not due to variation in visit rates.

Most bees landed on feeders, but a few bees landed on the tunnel floor or on the mesh covering the tunnel. If a bee continued to fly toward a feeder and landed on it, we included it in our data. We gave each bee 2 min to land on a feeder. We chose this time limit based on average flight time of around 30 s that our bees took to reach the feeder when it was placed at the 4.8 m tunnel position

during training. Any bee that landed elsewhere, or that failed to land within 2 min, was scored as not landing. We used a 2 min limit to test as many bees as possible before they became unmotivated due to waiting at the tunnel entrance. We refer to the setup of the feeders with a three-letter code that specifies color and position. For example, condition 7, in which there was a BR feeder at 4.8 m and a YS feeder at 7.2 m is abbreviated as “_BY” (the underscore refers to no feeder at the first position of 2.4 m).

Experiment 2: Novelty and motivation

From June to August of 2017, foraging honey bees from two different two-frame observation hives each containing approximately 2000 bees (von Frisch, 1967) were trained to a small, clear 2.0 mol/L unscented sucrose feeder in the early morning (around 8:00 to 9:00 am), and individually painted on the feeder. The target feeder was a transparent Petri dish (diameter 4 cm, height 1 cm) covered with aluminum mesh and filled with sucrose solution placed on top of a 1 m high tripod. We did not use odors during training to emphasize the visual features of the large local landmark: a bright blue umbrella (Wondershade™, Portable Umbrella, Royal Blue, Model number: MEWS21A-WS2RY) 1.5 m radius, height set to approximately 1.8 m, Fig. 3A) that accompanied the feeder throughout the training phase, and considerably changed the panorama of the foraging grounds due to its conspicuous height and color. In preliminary trials, we found that bees could easily learn the appearance of this blue umbrella and use it as a landmark in an otherwise relatively visually monotonous environment, a dirt road bordered by a chain link fence on one side and by bushes of approximately the same height and foliar density on the other side. We individually marked and trained bees to a site 80 m from the focal colony and recorded the number of times each bee visited the feeder within each 15 min period. We also noted if bees immediately landed or hesitated when they came within 1 m of the feeder. At the observation hive, a second observer counted the number of dance circuits each bee performed for the food source.

The experiment consisted of a before-move phase (2 h) and an after-move phase (1.5 h). At the beginning of the after-move phase, we separated the large landmark from the feeder by 6 m, a distance far enough to confuse the bees but not discourage them from searching for the feeder around the umbrella. This is a very short distance relative to the normal flight distances (> 6 km) of a foraging honey bees, (Beekman & Ratnieks, 2000), and therefore likely incurred only a slight increase in energy expenditure. We either moved the umbrella (large local landmark) to the side and retained the feeder and tripod (target) at the training position or kept the umbrella at the training position and moved the feeder and tripod to the side (Fig. 3B). Of the twenty-one bees that were successfully trained and continued to visit and dance, ten were from colony 1 and eleven from colony 2.

Statistical analyses

Experiment 1: Visual properties of a target versus its distance

To calculate the probabilities of landing if bees matched target image (shape and color) or distance, we used a multinomial model (Riefer & Batchelder, 1988) with the following assumptions. First, on each feeder bees can decide to land or not land. Second, to simplify the model, bees were assumed to land (or not land) only at three locations: 2.4 m, 4.8 m, and 7.2 m. At each location, the bee checks if the distance or the target match its prior memory and then lands with a certain probability. We calculated a third possibility, which is a baseline tendency of bees to land at any given spot, because bees may tend to land on any conspicuous object. An example decision tree is shown in Figure 2.

We built a decision tree for each test condition (Fig. S1) and ran simulations with different parameter level combinations to optimize our estimates of landing probabilities based upon whether bees used target image or distance memory. Each of our three parameters were tested in the range [0-1] with intervals of 0.025, resulting in $41 \times 41 \times 41 = 68921$ parameter combinations. For each

combination, we simulated 20 model bees that behaved with the given probabilities for each of our conditions (except condition 0, the color control). We then calculated the difference between our model and actual results for each condition and used a cost function (sum of squares of distances over all conditions) to assess the accuracy of the estimated behavior for each parameter combination. We then chose the combination (out of 68921 combinations) that minimized the cost function. We repeated this process 1000 times and obtained the distribution of each of our three parameters. Our experimental conditions were designed to enhance the estimation power of our model by creating highly different decision trees (Fig. S1) for the bees. For bees that landed within our 2-min interval, we compared the duration of flights between different conditions with Student's *t* test. We applied the Dunn-Sidak correction ($k = 2$) for multiple tests with the same data, denoting results that pass this test as “^{DS}”. We used the R software, version 3.4.3 (R Development Core Team) to build this model and to run all statistical tests. Graph 6b was drawn using package ggplot2, and all other graphs and statistical tests were performed using the core package.

Experiment 2: Novelty and motivation

To focus on the effect of the separation, we looked at the eight visits before and eight visits after moving the landmark. We chose a limited set of visits to equitably compare the before and after behavior of each bee, given that all bees did not perform the same number of visits before and after separating the landmark. We selected eight visits because it corresponds to 1 h, on average, provides a sufficient number of data points per bee for analyses. Other studies (De Marco & Farina, 2001; Gil *et al.*, 2007) show that bees can change their dance effort rapidly and they can learn reward amounts and rapidly change their foraging behavior accordingly. We expect flexibility in dance behavior to be rapid, otherwise it will may be of limited use to the dance followers.

We compared the number dance circuits per visit performed by bees (averaged per bee) before and after the separation of the large landmark and the target with a Repeated Measures Analysis of Variance (ANOVA) in R. In this model, the number of each bee's dance circuits for each visit was the

repeated measure (each bee is a subject with multiple visits), and type of separation (feeder moved away or landmark moved away) and experimental phase (before or after separation) were fixed effects. Visit rates in different 15 min periods were compared with a Repeated Measures ANOVA, with the 15-min period being the repeated measure for each bee, and time as a fixed effect.

Results

Experiment 1: Visual properties of a target versus its distance

As expected, bees preferred the correct target at the distance that they were trained to (4.8 m). In condition 3 (BY₋), bees seemed to fly over the blue feeder without even pausing to explore this newly-added, conspicuous object. However, bees relied upon visual memories of the target much more strongly than on distance memories when the visual attributes and the distance of the target were experimentally separated. Bees would land on a feeder with 0.88 probability if the shape and color matched their training period, independent of its distance from tunnel entrance. Conversely, their landing probability at the 4.8 m feeder was 0.25, regardless of the shape and color of the feeder. The baseline landing probability was low (0.10). Model parameters are shown in Table 3 and Figure 4.

Bee flight time in the tunnel was influenced by distance and the location and appearance of food sources along the flight path (Fig. 5). Bees spent less time flying when the feeder they landed on was closer to the entrance: comparing conditions 3 (BY₋) and 5 (YB₋) ($t = -3.93$, $df = 24.2$, $P = 0.0006^{DS}$) or comparing conditions 3 (BY₋) and 7 (₋BY) ($t = 1.56$, $df = 29.8$, $P = 0.13$). They also spent more time when they could not find a feeder associated with the correct feeder type: comparing conditions 6 (₋YY) and 8 (₋BB) ($t = 8.33$, $df = 8.6$, $P < 0.0001^{DS}$) or comparing conditions 2 (YY₋) and 4 (BB₋) ($t = -3.16$, $df = 10.1$, $P = 0.010^{DS}$).

The landing choice of the bees did not depend on the color and the shape of the targets they were trained to. Bees in condition 0 (trained to BR feeder and tested at “YB₋”) performed with

similar probabilities as other bees (Pearson's Chi-squared test, $\chi^2_4 = 6$, $P = 0.1991$). Flight time also did not differ between feeder types (YS or BR) because bees were equally able to find their training feeder, regardless of type, when the opposite type appeared along the way (conditions 0 (YB_) and 3 (BY_), $t = 0.10$, $df = 36.4$, $P = 0.92$).

Experiment 2: Novelty and motivation

The large local landmark (the umbrella) drew more attention from bees than the target (feeder and tripod). Even when the feeder stayed at the same location and the umbrella was moved away, bees always checked under the umbrella first, and, upon finding no feeder there, began to search at increasingly greater distances from the umbrella until they found the feeder. In the 15-min period immediately after landmark separation, bees had fewer visits to the feeder due to visiting the umbrella (effect of 15-min observation period on visit rate, $F_{2,36} = 3.74$, $P = 0.033$, $n = 19$). However, bees rapidly learned the new spatial relationship between the large landmark and the target and their visit rates returned to comparable levels ($t = -0.686$, $df = 109.7$, $P = 0.494$) after about 15-30 min (Fig. S3). Bees spent an average of 7.7 min searching after landmark separation, and this search time did not depend upon the type of separation ($t = -0.776$, $df = 13.8$, $P = 0.451$, $n = 18$).

Overall, bees changed their dancing effort when the target and the umbrella were separated (Fig. 6) but this was due to the temporary decrease in dancing immediately after the separation. Bees danced fewer circuits per trip in the period after separation, as shown in Figure 6A and 6C (effect of phase, $F_{1,19} = 5.03$, $P = 0.037$). It did not make a difference if the small inconspicuous target (feeder and tripod) was moved away from the large local landmark (umbrella) or *vice versa*, as shown in Figure 6C (effect of interaction of Phase \times Item Moved, $F_{1,19} = 0.86$, $P = 0.36$). However, this change was small (14% when feeder was moved away, and 12% when the umbrella was moved away).

There was variation in dancing motivation, but individuals were consistent. Bees that danced more before the move, also danced more after the move ($r = 0.832$, Fig. 6D). As expected, bees differed in terms of the number of dance circuits they produced (effect of bee identity, $F_{19,288} = 9.674$, $P < 0.0001$).

Discussion

Our results demonstrate that the reliance of bees on visual memories of the target is very strong, much stronger than their distance memories. We also show that changing a major landmark near a food source decreased their waggle dancing, likely due to decreased visitation because they consistently flew directly towards the major landmark. Foragers took longer to land when a food source's appearance, landmarks associated with it, or its location changed.

In experiment 1, bees showed a strong preference for the visual attributes of the food source, rather than its memorized spatial location as determined by its distance. We did not test for the effect of direction, which might also be an important stimulus and a focus for future studies. Considering the small amount of other visual features available to bees in the tunnels, due to absence of other landmarks, feeder shape and color were likely the only prominent characteristics that bees could rely on, making the feeder a prominent visual target. Tunnels provide amplified optic flow and their minimal design might push bees to use distance information to compensate for route images, but we did not see a strong effect of landing on the food source at the correct distance if it had the incorrect shape and color. Despite the intense optic flow, bees chose to ignore it when they had access to reliable visual cues from the target. Chittka & Geiger (1995) showed that bees not only measured distances but counted landmarks to learn where a feeder is located. Menzel *et al.* (2010) radar-tracked bees to test if bees use distance memories in conjunction with landmarks. They concluded that bees predominantly rely on the distance memory, although the number of landmarks on the path to the

foods source is also used by some bees. Srinivasan *et al.* (1997) showed that bees can use landmarks to make their navigational distance memory more accurate, but this does not necessarily translate into a more accurate waggle dance signal duration. Our experiments demonstrate that, when presented with highly conspicuous visual cues, bees primarily rely upon the shape and color if they must choose between the distance memory and image memory. It is important to note that in our experiment, as well as most related studies, researchers train bees from a hive to a given feeder by moving the feeder gradually away from the hive. This may motivate bees to rely on target shape and color instead of its location since having a moving feeder means that the location of the target is not associated with reward whereas its visual attributes are associated with reward at all times.

Bees rely on different cues at different parts of their route. Early stages depend mainly on the direction of flight, obtained from polarization patterns of the sky, whereas the later stages are guided by approaching specific landmarks and by the distance memory (Collett, 1996). Further investigation of spatial information of the target and its visual attributes can elucidate how and when each of these factors becomes the most reliable navigational cue, and the role of directional information. Open-field experiments would allow us to test these questions with a variety of distances and directions. However, the tunnel setup has the advantage of providing researchers with the opportunity to carefully observe each bee's exploration and landing choice(s) and to exclude the potential influencing presence of other foragers.

In experiment 2, our goal was to determine if local landmarks play a role in orientation (as in Tinbergen's 1935 classic landmark learning experiment with beewolf wasps) and also in determining the perceived profitability of a food source. A potential natural scenario would be the shifting or displacement of a major piece of foliage around a rewarding food source or emergence of a new flower. Trained foragers took only a few minutes of exploration near the major landmark to find the feeder. This behavior suggests that they form a visual memory from the feeder, but this information is not prioritized if the visual memory of a more conspicuous landmark (the large blue umbrella) can

guide them to the food source. This result does not mean that bees ignore direction and distance to return to a food patch, but that within a patch, landmarks are highly reliable as landing cues. Our results therefore reinforce prior studies demonstrating that bees rely on several types of landmarks for their navigation to rewarding food sources (Cartwright & Collett, 1982; Gould, 1996). They use large and conspicuous landmarks en route to or adjacent to the food source, and they also use the visual attributes of the resource itself.

Food quality and quantity (von Frisch, 1967; De Marco & Farina, 2001), food competition (Nieh, 2010), overcrowding (Lau & Nieh, 2010; Nieh, 1993; Thom *et al.*, 2003), and predator presence (Jack-McCollough & Nieh, 2015; Tan *et al.*, 2016) can all influence the number of waggle dance repetitions per forager. However, less is understood about whether waggle dancing by individuals will fluctuate when food sources are constantly profitable over long periods of time, since most studies have involved manipulating food quality or quantity (Raveret Richter & Waddington, 1993; De Marco *et al.*, 2005) rather than monitoring the long-term variations of individual bees' dance effort for an unchanging feeder. Our results show that dancers are mostly consistent over the duration of the day, but on average, changing the landmarks associated with a resource led to a short-term decrease in their dancing effort. This is likely due to the increased searching effort of foragers that did not find the food source next to the conspicuous landmark. This should decrease the profitability of the food source for that longer trip. Profitability depends primarily on the net energetic gain from the feeder, so even if the distance to the feeder is estimated to be higher due to high optic flow, shorter flight times elicit higher dance rates (Shafir & Barron, 2009). Alternatively, but not exclusively, the absence of a conspicuous landmark could lower a forager's motivation since receiving the reward at the food source is associated with approaching the landmark. There is considerable variation between different bees' number of waggle circuits per foraging trip, and the decline in dancing effort may not lead to a substantial change in recruitment.

Our experiment required us to use bees that were highly motivated to return to and dance for our food source during training and test phases. The dancing motivation of foragers can be quite variable (von Frisch, 1967), but foragers that are consistently motivated to dance are likely important colony sources of foraging signaling. The number of returns to the feeder did decline right after the separation, but this could be a result of bees taking longer to find the food source, not from a reduction in their willingness to search for the food.

These results suggest that familiar food sources are preferred to novel types of resources at a familiar location. Foragers must select between forming new spatial memories and forming new food-type preferences through new search images (Goulson, 2000), and the salience of each of these cues may play a role in the ontogeny of a forager's path throughout its lifetime. An interesting and open question is therefore how bees choose the landmarks they rely on, what exactly constitutes landmark conspicuousness, and how this influences navigation routes (Lindauer & Warwick, 1958; von Frisch, 1967).

Acknowledgments

We would like to thank Jessica Ma, William Bauer, Myra Julom, Richelle Vital, Brandon Hendrickson, Patrick Pasberg for their help with the experiments. Heather Broccard-Bell and Harmen Hendriksma and the comments of the anonymous reviewers significantly improved this manuscript. This research was supported by the UCSD Academic Senate, the Messier Grant, and a Sigma Xi grant.

Disclosure

The authors declare that they have no competing interests.

Data availability

All data will be available, upon publication, at zenodo.org.

Author contributions

BK and JCN conceived of and designed the experiments. BK, JC, and SG conducted the experiments. BK ran the data analyses. BK and JCN wrote the manuscript.

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Manuscript received May 25, 2018

Final version received September 26, 2018

Accepted October 16, 2018

Table 1 The locations and types of feeders used in testing the role of distance and visual attributes (shape and color) of the target feeder (experiment 1). We used either Yellow Square (YS) or Blue Round (BR) feeders. In each condition (except condition 1), two feeders are located at two out of three possible distances in the tunnel. The three-letter abbreviations are provided in the first column with the underscore indicating the position without a feeder. For example, YB_ references a condition in which the yellow and blue feeders are respectively at the beginning and middle positions with no feeder at the tunnel end.

Condition	Feeder at 2.4 m	Feeder at 4.8 m	Feeder at 7.2 m
0 (YB_)	YS	BR	No feeder
1 (_Y_)	No feeder	YS	No feeder
2 (YY_)	YS	YS	No feeder
3 (BY_)	BR	YS	No feeder
4 (BB_)	BR	BR	No feeder
5 (YB_)	YS	BR	No feeder
6 (_YY)	No feeder	YS	YS
7 (_BY)	No feeder	BR	YS
8 (_BB)	No feeder	BR	BR

Table 2 Where bees landed in tests of distance vs. target features (experiment 1). Each landing corresponds to a single bee trained to a yellow square (YS) feeder at 4.8 m except condition 0, in which bees were trained to a blue Round (BR) dish at 4.8 m) and tested in the presence of feeders indicated by the three-letter abbreviations. The three-letter abbreviations indicate feeder colors and positions. For example, “YB_” refers to a condition in which the yellow and blue feeders are respectively at the beginning (2.4 m) and middle (4.8 m) positions and no feeder is present at the end of tunnel (7.2 m). In condition 0, we tested 29 bees. In all other conditions, we tested 20 bees per condition.

Condition	Landing at the correct distance	Landing at the incorrect distance	No landing
0 (YB_)	16	8	5
1 (_Y_)	20	0	0
2 (YY_)	6	14	0
3 (BY_)	18	2	0
4 (BB_)	6	3	11
5 (YB_)	0	20	0
6 (_YY)	20	0	0
7 (_BY)	4	8	8
8 (_BB)	3	2	15

Table 3 The parameters of the distributions from our multinomial model for experiment 1. Each distribution consists of 1000 combinations of three parameters that resulted in the smallest cost function out of the 1000 iterations of all combinations.

Parameter	95% lower bound	Median	95% upper bound
$p(\text{Landing} \text{Baseline})$	0.000	0.100	0.200
$p(\text{Landing} \text{Correct Target})$	0.725	0.875	0.950
$p(\text{Landing} \text{Correct Distance})$	0.050	0.250	0.425

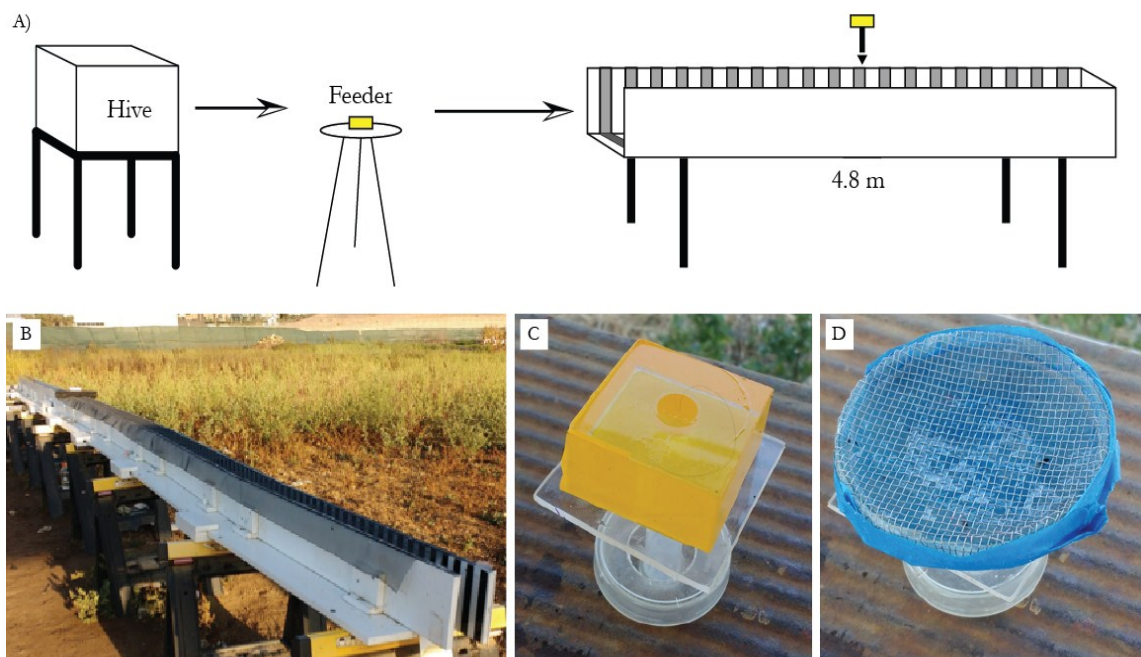


Fig. 1 The setup for testing the roles of distance vs. target image (experiment 1). (A) Bees were trained from each hive to the tunnel entrance and then into the tunnel. (B) View of the tunnel set-up with the mesh on top. (C) Yellow Square (YS) feeder. (D) Blue Round (BR) feeder. The transparent stands are set under the feeders when the feeders are in the tunnels and are identical.

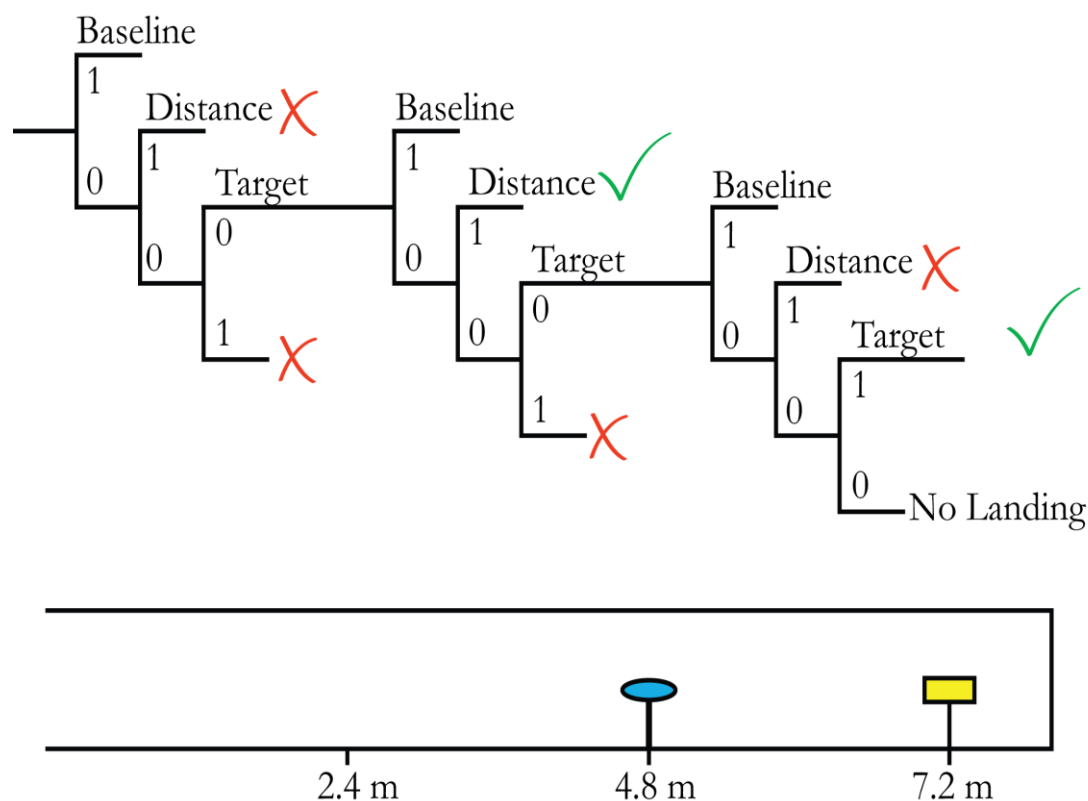


Fig. 2 Example decision tree for condition 7 (No feeder at 2.4 m, Blue Round feeder at 4.8 m and Yellow Square feeder at 7.2 m, abbreviated as “_BY”). As the bee flies through the tunnel, it passes over three distinct segments. In the model, the bee checks at each segment if it wants to land due to a baseline landing tendency (in all segments), if it wants to land due to a match between its distance memory and current distance (only at 4.8 m) or due to matching the target it has been trained to and the current feeder (any feeder that looks yellow and is cuboidal). Its landing because of a match is determined by the respective probability: $p(\text{Landing}|\text{Baseline})$, $p(\text{Landing}|\text{Correct Distance})$ and $p(\text{Landing}|\text{Correct Target})$. In this example, the bee ignores the first segment, ignores the BR feeder, and lands on the YS feeder.

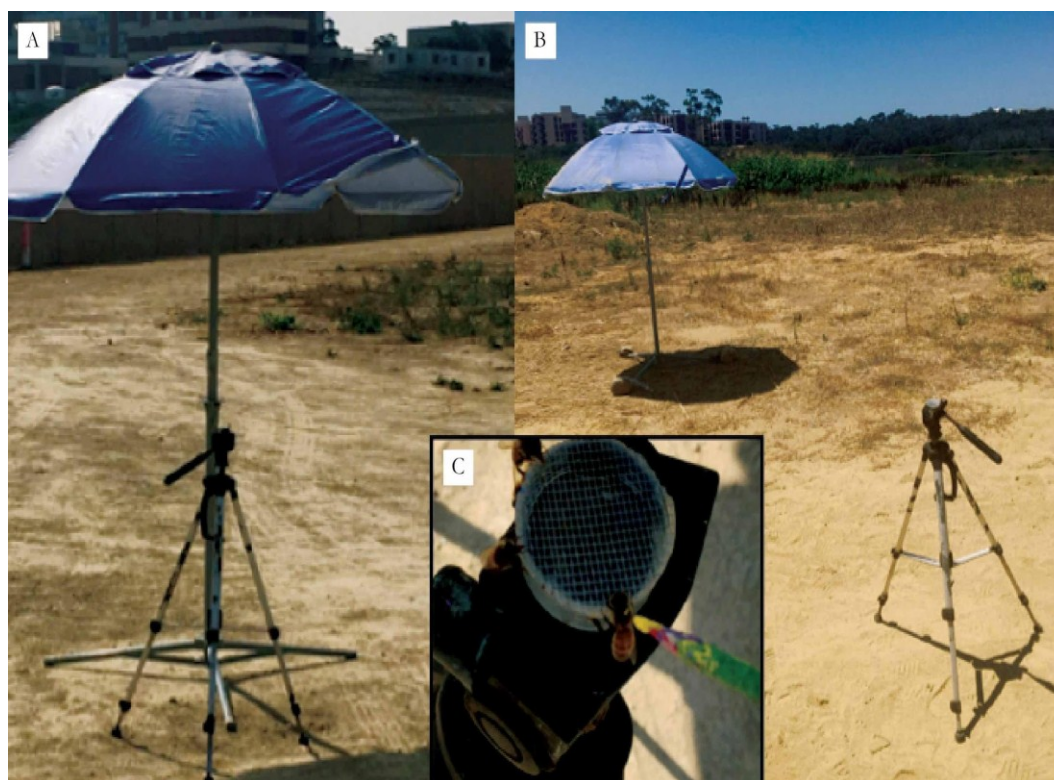


Fig. 3 The setup of the novelty and motivation experiment (experiment 2). (A) Before the separation and throughout the training phase, the major landmark (umbrella) is directly above the visual target (feeder on top of a tripod). (B) After the separation, the tripod and the feeder are at the same location, but the umbrella has been moved about 6 m away to the south of the feeder while bees fly east to get from their hive to the feeder. (C) Close-up showing bees on the unscented feeder. One bee is being marked with a yellow dot.

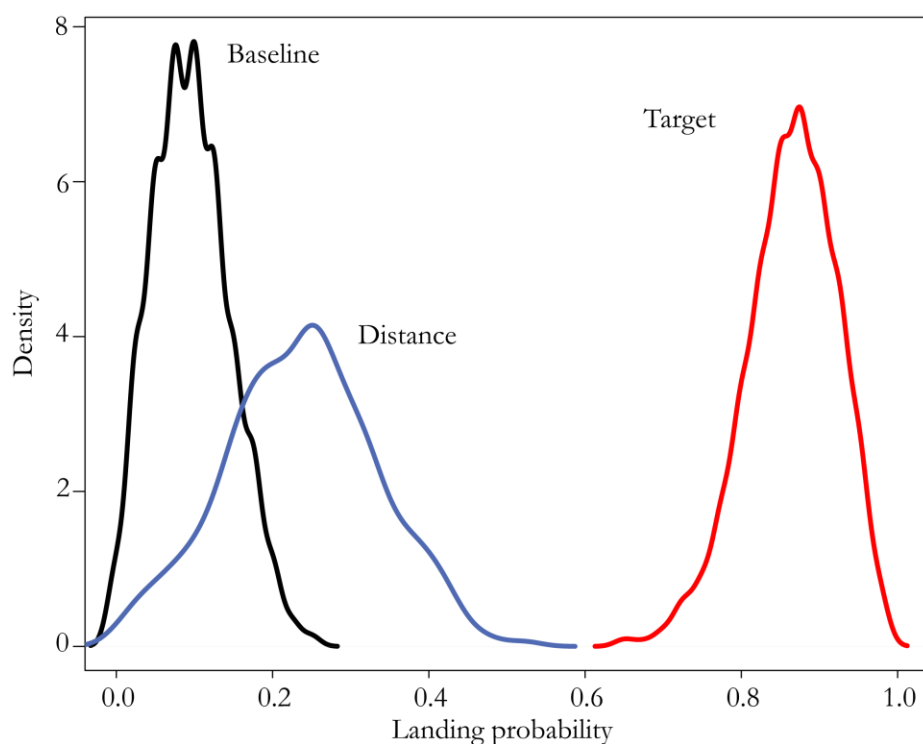


Fig. 4 Parameter distribution densities for each matching parameter's landing probability. The black line represents the distribution of the baseline landing probability anywhere in the tunnel. The green line shows the distribution of landing probability on the feeder at the correct distance. The red line represents the distribution of landing probability on the target feeder with the correct shape and color. The distributions are not completely smooth since our parameter ranges were quantized at intervals of 0.02.

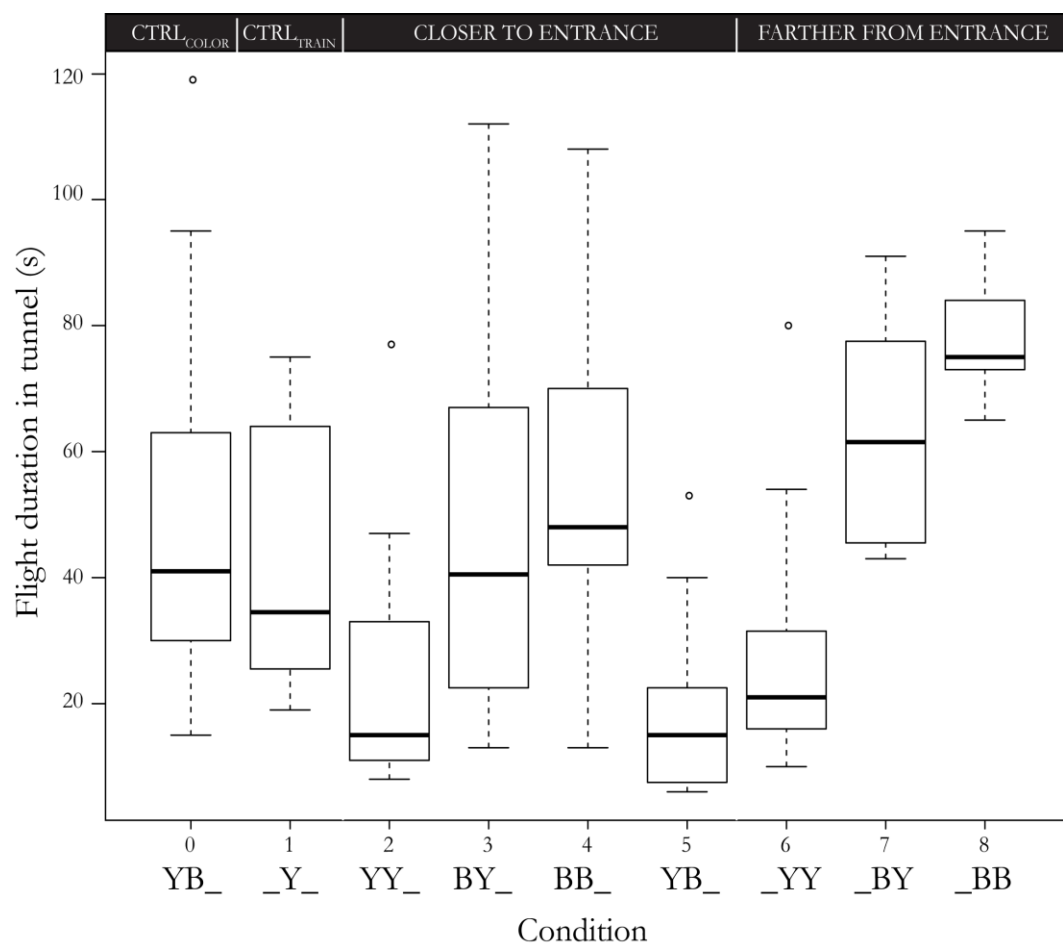


Fig. 5 Results of experiment 1: latency to land for each test condition. Bees took less time to travel down the tunnel and land on the feeders if the feeders were closer to the entrance and were of the same type as the training feeder. The three-letter abbreviations indicate feeder colors and positions. For example, “YB_” refers to a condition in which the yellow and blue feeders are respectively at the beginning (2.4 m) and middle (4.8 m) positions and no feeder is present at the end of tunnel (7.2 m). Condition 0 serves as a control for color bias of bees. Condition 1 serves as a control for landing affinity of bees. In conditions 2 through 5, an extra feeder is added at 2.4 m. In conditions 6 through 8, an extra feeder is added at 7.2 m. Table 2 lists the details of feeders, training methods, and landing choices.

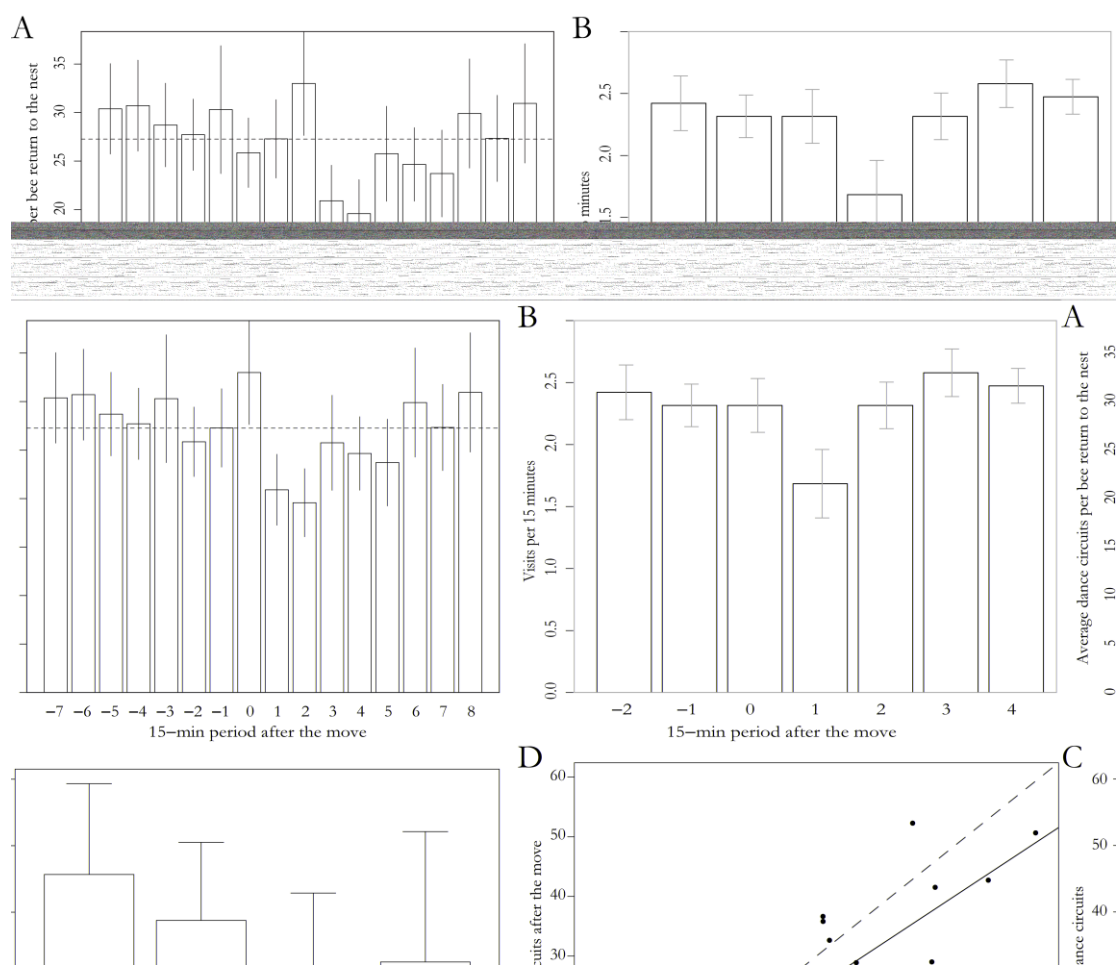


Fig. 6 Effects of landmark displacement on bee waggle dancing (experiment 2). (A) Dancing effort per trip declines as a result of separating the food source from the prominent landmark, but recovers after around 30 minutes. Separation occurs between periods 0 and 1. (B) The number of visits per 15-minute period declines right after the separation of the feeder and the umbrella. However, after 15 minutes, bees have learned the new location of the feeder and visits return to their previous levels. (C) The type of move (feeder or umbrella moved away) did not change alter the number of dance circuits ($n = 11$ bees for feeder moved and $n = 10$ bees for umbrella moved). (D) Bees that danced more circuits before the feeder was separated from the landmark also continued to dance more after the separation. However, 62% of bees danced fewer circuits after the move. Each dot represents one bee's average number of circuits before (on x axis) and after (on y axis) the separation. Solid line: regression line $y = 0.85x + 0.313$, Dashed line: identity line $y = x$.

Supplementary material

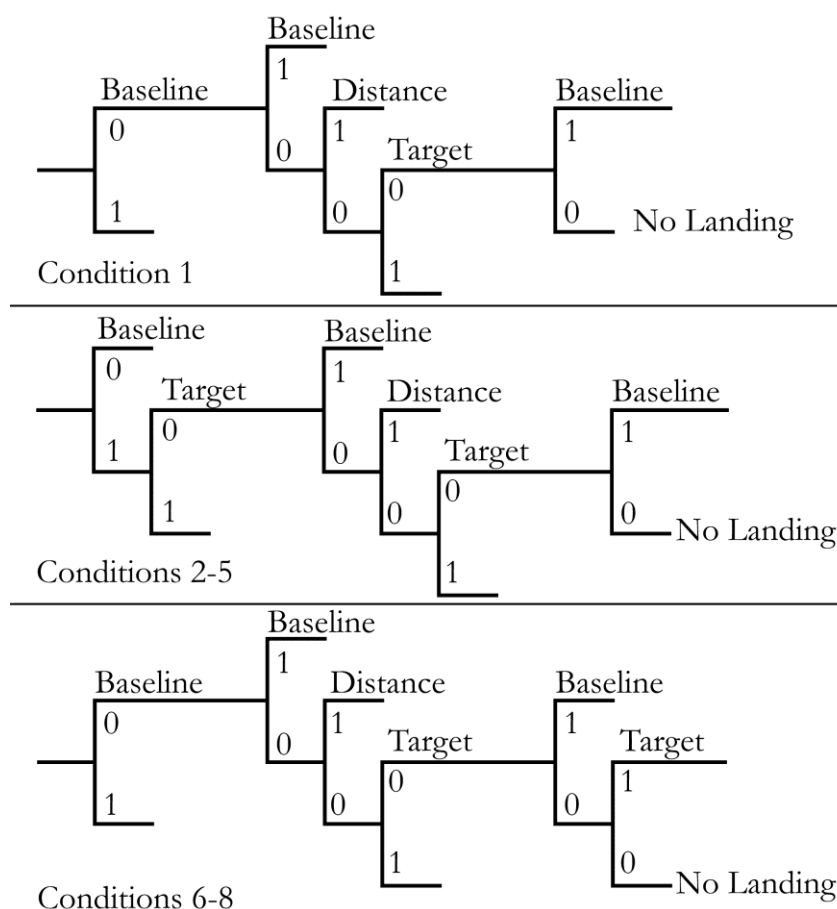


Fig. S1 Decision trees for each test condition in experiment 1. Table 2 lists the details of feeders, training methods, and landing choices. Condition 1 contains only one feeder similar to the one the bees have been trained to, at the location that bees have been trained to. In conditions 2 through 5, an extra feeder is added at 2.4 m and the feeder at the correct distance (4.8 m) is either kept the same or changed to a Blue Round feeder. In conditions 6 through 8, an extra feeder is added at 7.2 m and the feeder at the correct distance (4.8 m) is either kept the same or changed to a Blue Round feeder. All three potential feeder positions offer the Baseline landing, but only the middle feeder location (4.8 m) has the distance memory matching option. Depending on the condition, each feeder can offer a matching image (when it offers a Yellow Square feeder).

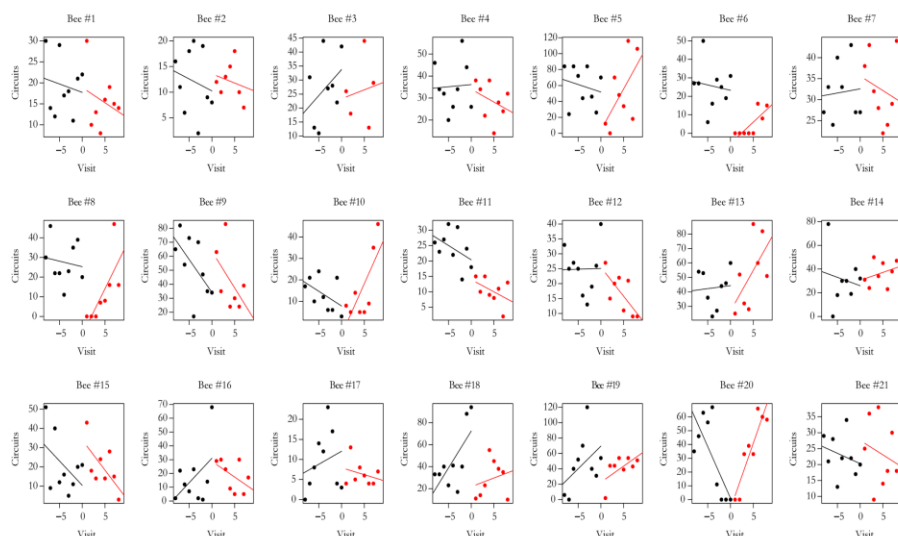


Fig. S2 The number of dance circuits by individual bees during each visit back to the nest before and after the major landmark was moved in experiment 2. The move occurred between visits 0 and 1 and thus negative and positive visits respectively show visits before and after the landmark was moved. The circuits danced for each visit is the sum of all dance circuits that a returning forager performed during her time in the hive before flying again to the feeder. A figure-8 movement thus would count as two circuits. Black dots refer to the circuits danced after each visit prior to the separation of the major landmark from the feeder, and the black line is the linear regression for those dots. Similarly, red dots refer to the circuits danced after each visit following the separation of the major landmark from the feeder, and the red line is the linear regression for those dots.