



Honeybee navigation: odometry with monocular input

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ABSTRACT

Recent studies have revealed that navigating honeybees, *Apis mellifera*, estimate the distance to a food source by integrating over time the image motion that they experience en route. Here we examine the ability of honeybees to gauge distance travelled when visual input is available primarily to one eye. Bees were trained to fly into a tunnel, lined with textured patterns, to collect a reward at a feeder placed at a certain distance. Their ability to estimate distance flown was then assessed by testing them in a fresh tunnel without the feeder. The results show that (1) bees can estimate distance flown under monocular conditions, performing nearly as accurately as when information is available to both eyes; (2) bees can learn to fly two different distances, where each distance is measured in terms of the image motion experienced by a different eye; and (3) bees that have acquired information on the distance to a food source using one eye can measure out the same distance when they are required to use the other (naive) eye. The need to measure distance using signals from a single eye becomes important when a bee flies to a food source along the face of a cliff or the edge of a forest. Furthermore, under such conditions, it is important to be able to deal with odometric signals that are transposed interocularly when the bee returns home from the food source. This is because, although distances are learnt primarily on the way to a food source, foraging bees monitor distance flown on the homebound as well as the outbound routes.

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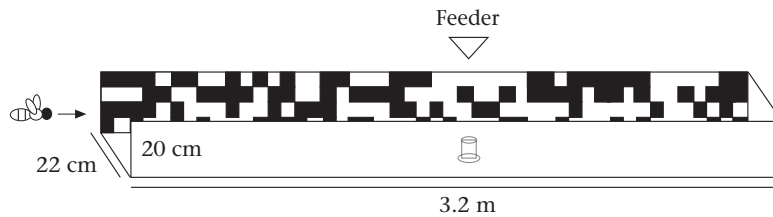
Honeybees, *Apis mellifera*, fly up to several kilometres to collect pollen or nectar. To find her way to a food source and back to the hive, a bee needs to know the direction in which she is flying, as well as the distance that she has covered. It is well established that bees determine their flight direction by using cues derived from the celestial compass (reviewed by Wehner 1992). However, the cues by which they gauge the distance flown to the goal have been the subject of controversy ever since the pioneering work by Karl von Frisch (reviewed by von Frisch 1993, pp. 109–129). Early studies suggested that this distance is measured in terms of the energy expended during flight (reviewed by von Frisch 1993; Heran & Wanke 1952; Heran 1956), but recent findings (Neese 1988; reviewed by Esch & Burns 1996) question this hypothesis. They suggest, instead, that an important cue for gauging the distance flown is the amount of image motion, integrated over time, experienced by the bee on the way to the goal. This conclusion appears to hold at least for foraging

distances ranging from a few metres (Schöne 1996; Srinivasan et al. 1996, 1997) to ca. 100 m (Esch & Burns 1995, 1996). It is also supported by the studies of Ugolini (1987) in which wasps, *Polistes gallicus*, were passively displaced from their nest in test tubes, and then tested for their ability to return home. Insects that enjoyed a view of the environment during the displacement were able to fly home successfully, whereas insects that were deprived of this view were not.

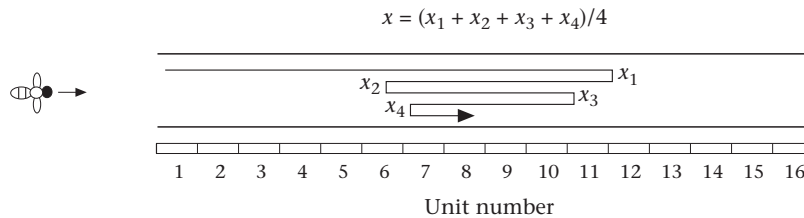
There are several lines of evidence that indicate that information on the distance to a food site is acquired primarily on the outbound route (reviewed by von Frisch 1993, pp. 116–121; Heran & Wanke 1952; Heran 1956; Srinivasan et al. 1997; but see Otto 1959). However, the bee also needs (and uses) distance information to navigate her way back to the hive, when she flies in the opposite direction (Srinivasan et al. 1997). When the two eyes experience equal amounts of image motion en route, then the odometric (distance) information acquired during the outbound route can be directly applied to the homing trip as well, because the image motion seen by each eye will not depend on the direction in which the bee flies. Under natural conditions, however, the two eyes would rarely experience the same amount of image motion on the way to the feeding site, unless the

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(a) Training



(b) Food-search test



(c) Homing test

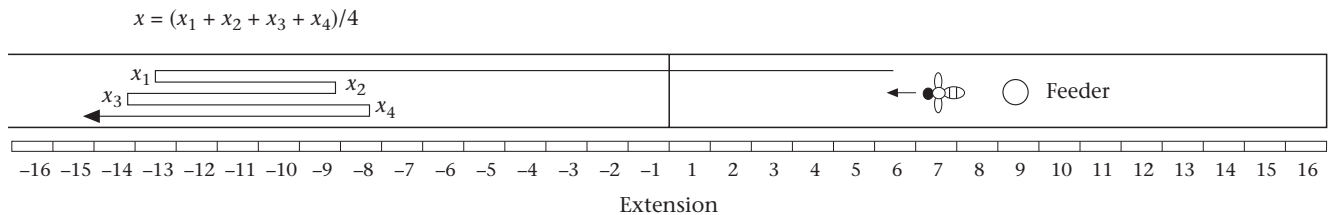


Figure 1. General layout of training and test situations. Bees, trained to find a food reward (circle) placed at a specific location in a training tunnel (a), were subsequently tested in two situations. In the 'food-search test' (b), the trained bees encountered a fresh tunnel identical to the training tunnel, which carried no reward. In the 'homing test' (c), the reward was retained, as in the training situation, but an extension was apposed to the entrance of the original training tunnel after a bee had alighted on the feeder. In either case, the behaviour of the bee as it searched for the feeder (food-search test) or for the tunnel exit (homing test) was recorded and analysed as described in the General Methods.

environment is perfectly symmetrical with respect to the two eyes. Thus, under natural conditions, the odometric mechanism would have to be flexible enough to cope with the transposed signals received by the two eyes on the homeward journey.

We examined whether, and how well, bees can estimate distance travelled when information on image motion is confined primarily to one eye. We also asked whether bees can learn to associate the different patterns of image motion that are likely to be experienced on different routes, with different flight distances. This ability is likely to be important when a given bee forages at two different sites (depending, for example, on the time of day; reviewed by Menzel 1990). Very different patterns of stimulation would occur, for example, when the route to one feeding site runs parallel to a cliff on the left-hand side, and the route to the other site runs parallel to the edge of a forest on the right-hand side. A bee flying these two routes must be able to measure out the distance along one route with one eye, and the distance along the other route with the other eye. We examined whether bees can cope with such a task. Next, we investigated interocular flexibility in distance measurement by asking whether bees that have acquired information on the distance to a

food source using one eye can measure out the same distance when they are required to use the other (naive) eye. Finally, we explored the real-life relevance of such interocular flexibility by investigating whether bees that have learned the distance to a food site by using one eye can monitor distance flown on the way home by using the other eye.

GENERAL METHODS

In each experiment (unless otherwise specified), 10–15 bees were marked with coloured dots (Chrome Acrylics Pty. Ltd, Sydney, Australia) on the thorax and abdomen so that they could be individually identified. They were trained in a large indoor flight room to fly into a tunnel measuring 3.2 m long, 22 cm wide and 20 cm high, to find a feeder containing sugar water placed at a fixed location (Fig. 1a). In some experiments, both walls of the tunnel were lined with a randomly textured, black-and-white Julesz pattern of pixel size 1 cm, in which case both walls provided the flying bee with equal amounts of image motion. In other experiments, only one of the walls was lined with the random texture; the other wall was lined with axially oriented, black-and-white stripes of

period 4 cm, or was homogeneously white, and thus offered no motion cues. Unless otherwise specified, the floor of the tunnel was homogeneously white. The tunnel was covered by a transparent ceiling of Perspex which forced the bees to stay inside the tunnel on the way to the feeder and back. A white cloth canopy, suspended 1.5 m above the tunnel, screened the bees' view from potential landmarks in the ceiling of the flight room. During training, the position and orientation of the tunnel within the flight room were varied frequently to prevent the bees from using any residual external landmark information to gauge their position in the tunnel. We know, from earlier work, that bees trained in this way learn the distance to the reward by integrating, over time, the image motion that they experience on the way to the feeder (Srinivasan et al. 1996, 1997). In all of the experiments, training was carried out for at least 2 full days, encompassing about 150 rewards per bee, on average, before testing was commenced.

After training, we tested the bees individually in two kinds of experimental situations. In one test situation, termed 'food-search test', the trained bees encountered a fresh tunnel identical to the training tunnel, in which the feeder was absent (Fig. 1b). In the second test situation, termed 'homing test', the reward was retained, as in the training situation, but an extension was apposed to the entrance of the original training tunnel after a bee had alighted on the feeder (Fig. 1c). Food-search tests were conducted in all of the experiments. They assessed the bees' capacity to measure the distance flown from the tunnel entrance to the feeder. Homing tests were conducted in only some of the experiments. They assessed the bees' capacity to measure the distance flown on the way back, that is, from the feeder to the tunnel exit. In either case, we recorded the behaviour of the bee as it searched for the feeder (in the food-search tests) or for the tunnel exit (in the homing tests). We analysed the data as described below.

In a test, a bee typically flew back and forth along the tunnel, making a number of U-turns as it searched for the reward (food-search tests) or the tunnel exit (homing tests). For the purposes of analysis, we subdivided the tunnels into units, each 20 cm long (Fig. 1b, c). In the tests, we quantified the bee's searching behaviour by visually recording the unit numbers x_1 , x_2 , x_3 and x_4 in which she made the first, second, third and fourth U-turns, respectively. The average of these four values, \bar{x} , provided an estimate of the bee's mean search position in the test under consideration.

For each test, we calculated the mean search position and its standard deviation from the values of x (see above), measured for a number of flights (N). In the figures, the mean search position is depicted by the vertical dashed line. We used Student's t tests to test for statistical significance of the difference between the expected and the experimentally measured mean search positions, as well as the difference between the mean search positions measured in different experiments. We used the F test (Snedecor & Cochran 1967) to test for significant differences in standard deviation of the mean search position between experiments.

We characterized the spatial distribution of the search (search distribution), shown as curves in the figures, by measuring the number of times the bee entered each unit during the period over which the first four U-turns were made (for further details see Srinivasan et al. 1996). The spatial distribution of the positions of the first U-turns (x_1) was also computed and is shown as histograms in the figures.

The mean search position and its standard deviation are probably the best measures of the accuracy and precision of the bee's odometric mechanism. The spatial distribution of the positions of the first U-turns also provides a measure of precision, but, since bees tend to overshoot the position of the reward when it is absent, this distribution is likely to be skewed towards longer distances. The search distribution, on the other hand, is not likely to be skewed in this way. The standard deviation of the mean search position is likely to be smaller than the half-width of the search distribution because, while the former is a measure of the precision of the odometer, the latter is a measure of the 'spread' of the bees' searching effort.

We carried out tests for brief periods (ca. 20 min), testing several individual bees in each of these periods. Between successive test periods, training was continued for at least 2 h. Tests of a given type were carried out several times, in order to obtain sufficient data (number of flights, N).

METHODS AND RESULTS

Experiment 1: Monocular Distance Estimation

We began by asking how well the bee's visual odometer functions when information on image motion is restricted almost exclusively to one eye. One group of bees was trained in a tunnel in which both walls as well as the floor were lined with a random texture (Fig. 2a). Bees entering this tunnel experienced image motion in both eyes. Another group of bees was trained in a tunnel in which the right wall carried a random texture, the left wall carried axial stripes, and the floor was homogeneously white (Fig. 2b). Bees entering this tunnel experienced image motion primarily in the right eye. The left eye did not participate in acquiring distance information. (Earlier work, outlined in the Introduction, has established that distance is learnt only on the way to the food source.)

In each case, the feeder was positioned in the centre of unit 9 (i.e. at a distance of 170 cm from the tunnel entrance). After training, we conducted food-search tests with bees of both groups. In both of these experiments, the mean search position in the tests was not significantly different from that corresponding to the location of the feeder during training. Clearly, the bees were able to keep track of how far they had travelled even when deprived of image motion in one eye (Fig. 2b). Moreover, the standard deviations of the mean search positions (details in figure legend) were also not significantly different in the two conditions, indicating that the precision with which the bees judged distance travelled was not impaired by depriving one eye of image motion. However, the search

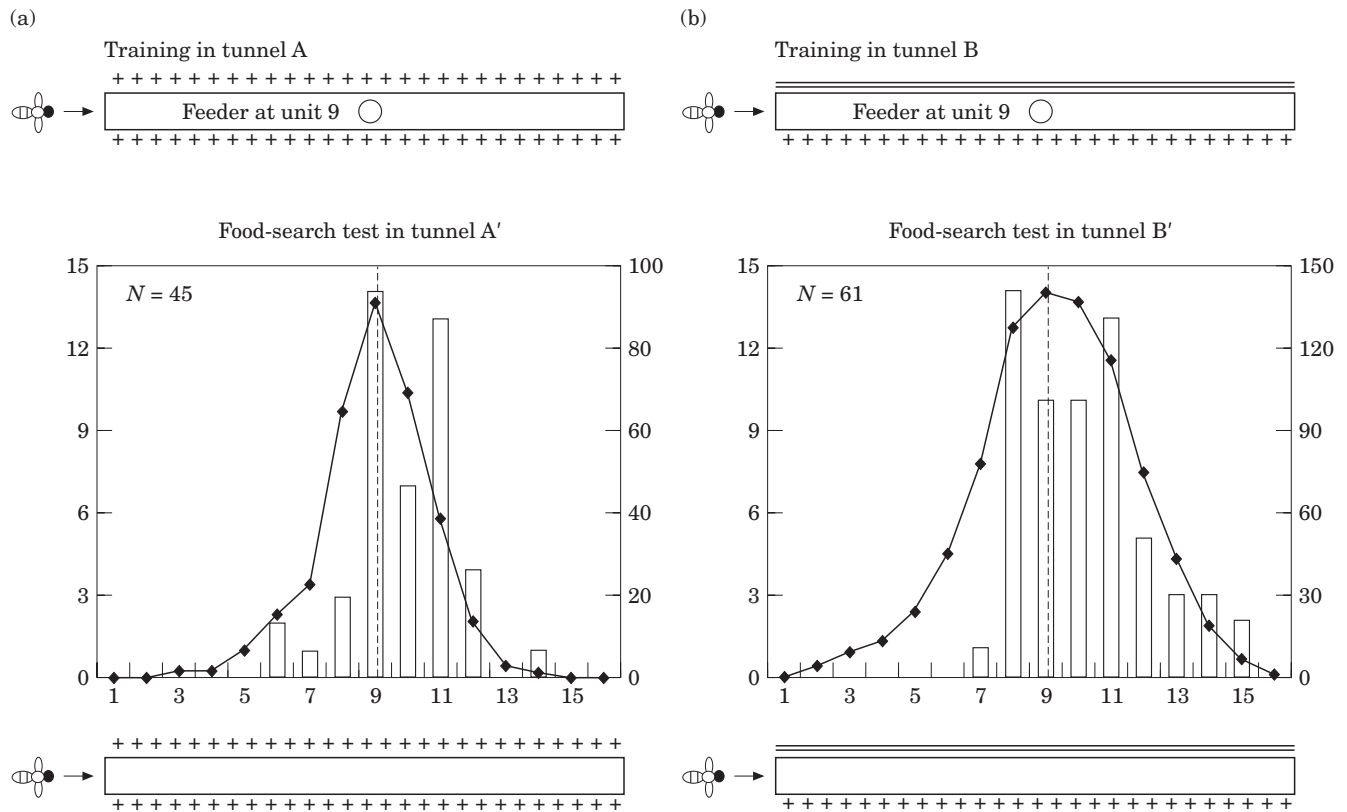


Figure 2. Experiment comparing the ability of bees to estimate distance travelled when odometric information is available (a) to both eyes, and (b) to only one eye. In (a), bees were trained in a tunnel (A) in which both walls as well as the floor were lined with a random texture (+++); in (b), another group of bees was trained in a tunnel (B) in which the right wall carried a random texture, the left wall carried axial stripes (=), and the floor was homogeneously white. In each case, the feeder was positioned at a distance of 9 units from the tunnel entrance. In subsequent food-search tests, the bees were presented with identical tunnels (A' or B'), with the reward absent. The dashed vertical line depicts the mean search position, the curve the search distribution (right ordinate, arbitrary units) and the histogram the distribution of the first U-turns of bees entering each tunnel (left ordinate, arbitrary units; details in General Methods). In tunnel A', the mean search position \pm SD is at 9.06 ± 1.06 units; in tunnel B', it is at 9.26 ± 1.00 units. The mean search positions and their standard deviations were not significantly different in the two tunnels (means: $P > 0.80$; SDs: $F = 1.12$ ($< 1.22 = F_{0.25}(44,60)$), $P > 0.25$, F test for variance). Furthermore, neither mean search position was significantly different from the position of the reward during training ($P > 0.50$ in either case). N = number of flights evaluated.

distribution was somewhat broader with monocular stimulation (compare Fig. 2b with Fig. 2a), suggesting that the bees tended to focus their search more sharply when both eyes participated.

We repeated the above experiment with the feeder placed at a different distance from the tunnel entrance: at the centre of unit 4 (Fig. 3). Very similar results were obtained (details in legend).

The spatial distribution of the positions of the first U-turns (see histograms) in these and subsequent experiments indicates that, in the tests, bees tended to make their first U-turn near the former location of the feeder, or soon after they had passed that location. This would seem to be a reasonable searching strategy; we return to this point in the Discussion.

Experiment 2: Estimation of Distances with Two Eyes

We then asked whether bees can learn two different distances, where each distance is measured in terms of the

image motion experienced by a different eye. A group of bees was trained to fly alternately into two different tunnels, A and B (Fig. 4) that were interchanged every 15 min, corresponding to three visits per bee, on average. Only one tunnel was presented to the bees at any given time. Tunnel A carried a random texture on the left wall and axial stripes on the right wall. Thus, a bee entering this tunnel experienced image motion primarily in the left eye as she flew to the feeder. Tunnel B was lined with the axial stripes on the left wall and the random texture on the right wall. A bee entering this tunnel experienced image motion primarily in the right eye on the way in. Tunnel A offered a reward at the centre of unit 9; tunnel B offered a reward at the centre of unit 4.

After training, food-search tests were conducted in an identical, but fresh set of tunnels, A' and B'. Only one of the tunnels was presented in any given test. As Fig. 4 shows, the bees were able to distinguish between the two tunnels, and to search for the reward at a distance that was appropriate to each tunnel. Furthermore, they measured distance flown by using image motion

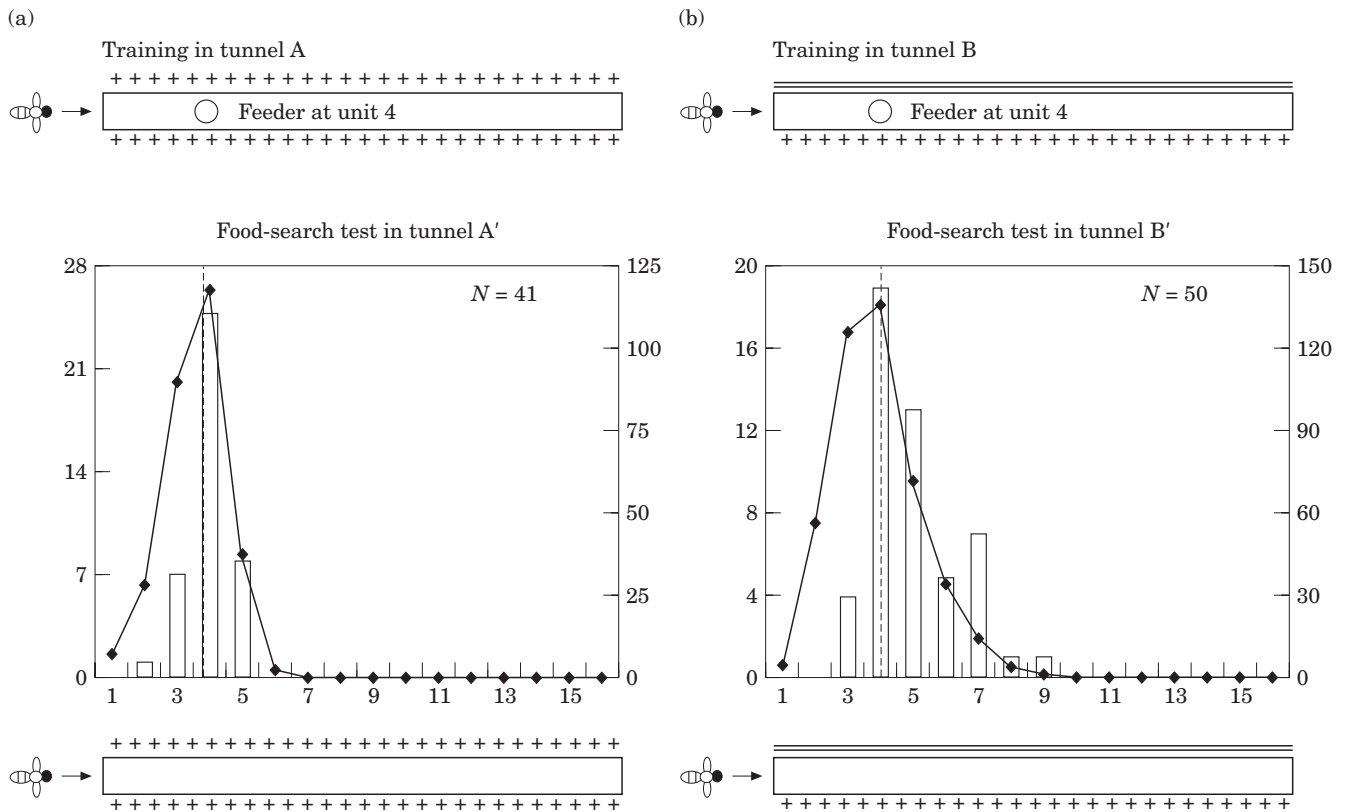


Figure 3. Experiment similar to that described in Fig. 2, but with the feeder placed at the centre of unit 4. In food-search tests, bees searched at a mean position \pm SD of 3.59 ± 0.62 units in tunnel A' and at 3.70 ± 0.69 units in tunnel B'. The mean search positions and their standard deviations were not significantly different in the two tunnels (means: $P > 0.40$; SDs: $F = 1.24$ ($< 1.49 = F_{0.1}$ (49,40)), $P > 0.10$, F test for variance). Other details as in Fig. 2.

experienced primarily by the left eye while in tunnel A', and primarily by the right eye while in tunnel B'. Thus, bees can use each eye to measure a different distance.

Experiment 3: Interocular Transfer of Learned Distance

The above finding raises the possibility that bees, whilst flying to a food source, might acquire and remember odometric information in a rather rigid way, by retaining separately the information on the image motions experienced en route by each eye. Is this really the case, or is there some plasticity in honeybee odometry?

To address this question we examined whether bees, trained to find a feeder at a fixed position in a tunnel by measuring distance through image motion that is available to only one eye, can measure out this distance when image motion is presented to the other, naive eye (the eye that had not experienced the image motion). Initially, we attempted to do this experiment by training bees to enter a tunnel with a feeder placed at a fixed distance from the entrance. The tunnel carried a random texture on the left wall and axial stripes on the right wall. After training, we attempted to test the bees by confronting them with a fresh tunnel in which the random texture was on the right wall, and the axial stripes on the left wall. However, bees trained in this way refused to enter the test tunnel.

Evidently, the bee's pattern recognition mechanisms signalled an unfamiliar tunnel where a reward was not to be expected. Therefore, we used a modified training procedure in which the bees were trained simultaneously in two tunnels, one at a time, alternating every 15 min (corresponding to three rewarded visits per bee, on average).

One tunnel (A) carried a random texture on the left wall and axial stripes on the right wall; it offered a reward at a distance of 6 units from the entrance (Fig. 5a). The other tunnel (B) carried a random texture on the right wall and axial stripes on the left wall. It offered a reward at a randomly varying distance from its entrance (this distance was determined afresh by a random-number sequence every time tunnel B was presented). In the food-search tests (Fig. 5b, c), the bees were offered fresh tunnels A' and B', identical to A and B, again one at a time.

If the bees had learnt the tasks presented to them in the two tunnels, they should search at a distance of 6 units from the entrance in tunnel A', and randomly (i.e. over the entire length) in tunnel B'. The results (Fig. 5b, c) show that, in tunnel A', the mean searching location and the peak of the search distribution were indeed close to unit 6, as expected. However, the searching distribution in tunnel B' was not random: it was again concentrated in the vicinity of unit 6. In fact, the shape of the search

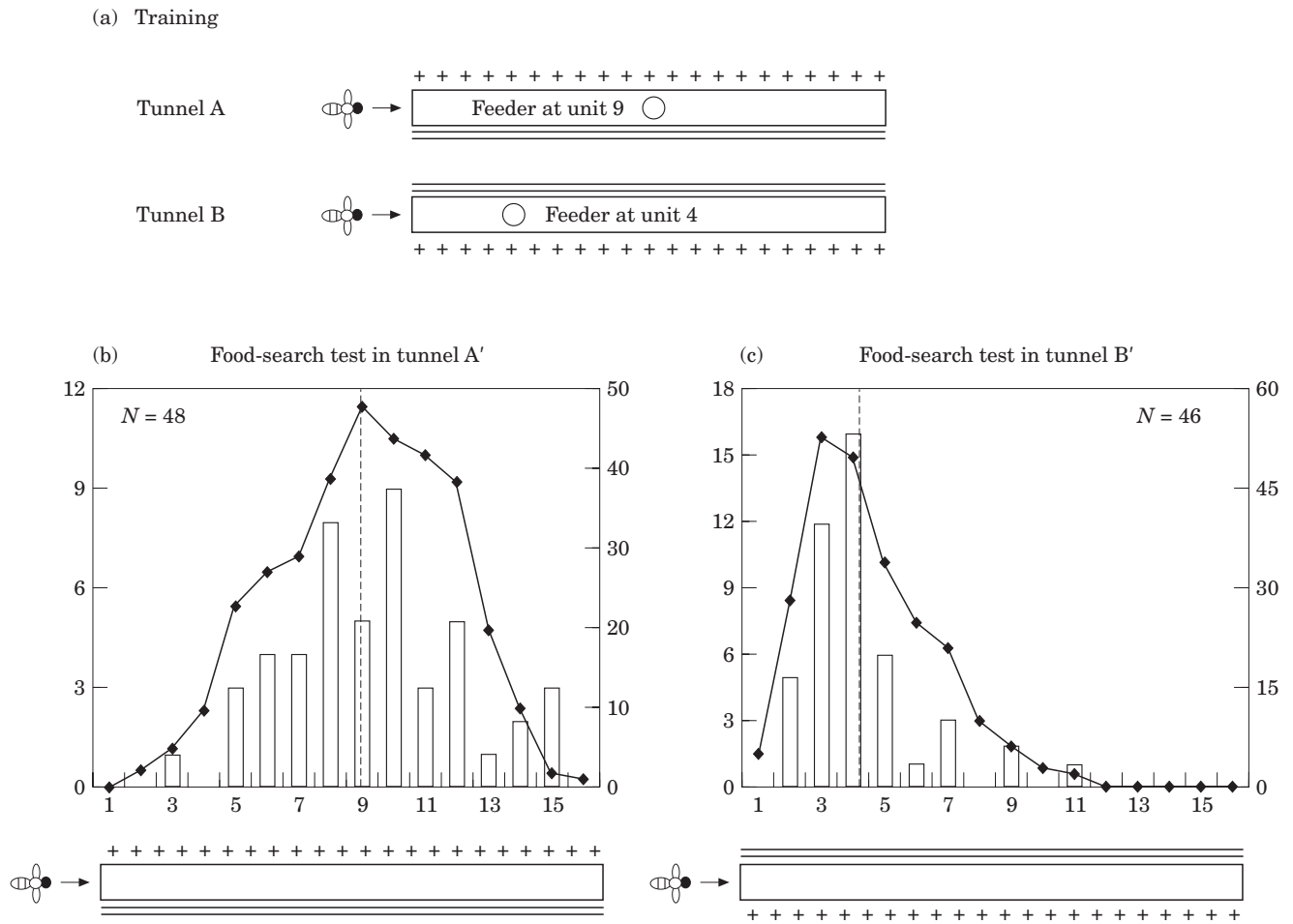


Figure 4. Experiment to investigate whether bees can simultaneously learn to measure two different flight distances with the two eyes. (a) Training. In tunnel A, lined with a random texture on the left wall and axial stripes on the right wall, the feeder was positioned 9 units from the entrance; in tunnel B, lined with a random texture on the right wall and axial stripes on the left wall, the feeder was at a distance of 4 units. The lower panels show the performance of the bees in food-search tests in a tunnel A' identical to A (b) and a tunnel B' identical to B (c). In tunnel A', the mean search position \pm SD was at 8.95 ± 2.39 units (dashed vertical line); in tunnel B', it was at 4.15 ± 1.5 units. In each case, the mean search position was not significantly different from that corresponding to the location of the reward in the appropriate training tunnel (Case 1: $t_{47} = 0.14$, $P > 0.80$; Case 2, $t_{45} = 0.68$, $P > 0.50$). The mean search positions differed significantly between the two test tunnels ($t_{92} = 11.60$, $P < 0.001$). Other details as in Fig. 2.

distribution in tunnel B' was very similar to that in tunnel A'. Evidently, the bees, which had learnt to fly a distance of 6 units into tunnel A by integrating image motion in the left eye, were integrating the image motion provided by the right eye when they entered tunnel B' and used this information to fly a distance of 6 units even in this tunnel.

We repeated the above experiment with a fresh group of bees, training them to a different distance in tunnel A: this time the feeder was positioned 11 units from the entrance (Fig. 6a). The position of the feeder in tunnel B was varied randomly, as before. In the food-search tests (Fig. 6b, c), the trained bees searched at a mean distance close to 11 units in both test tunnels, A' and B', replicating the findings of the previous experiment.

These results reveal that bees that have been trained to use image motion in one eye to fly a specific distance can use image motion in the other eye to fly the same distance.

To reinforce the validity of this conclusion, we carried out a control experiment (Fig. 7) in which bees were trained to fly into a single tunnel with a random texture on the right wall and axial stripes on the left wall (Fig. 7a), as in tunnel B of the experiments of Figs 5 and 6. The position of the feeder in the tunnel was varied randomly every 15 min (corresponding to three rewarded visits per bee, on average). When bees trained in this way were subjected to a food-search test they displayed a broad search distribution, characteristic of a nearly random searching pattern (Fig. 7b). This experiment shows that, when the feeder is not at a fixed distance, bees do not show a tendency to search at a specific location. Thus, the peaked distributions that the bees showed in the B' test tunnels of the experiments shown in Figs 5 and 6 cannot be due to any extraneous experimental artefacts. They are a direct consequence of training in the A tunnels with the feeder at a fixed distance, and of the bees' ability to read out this distance even with the naive eye.

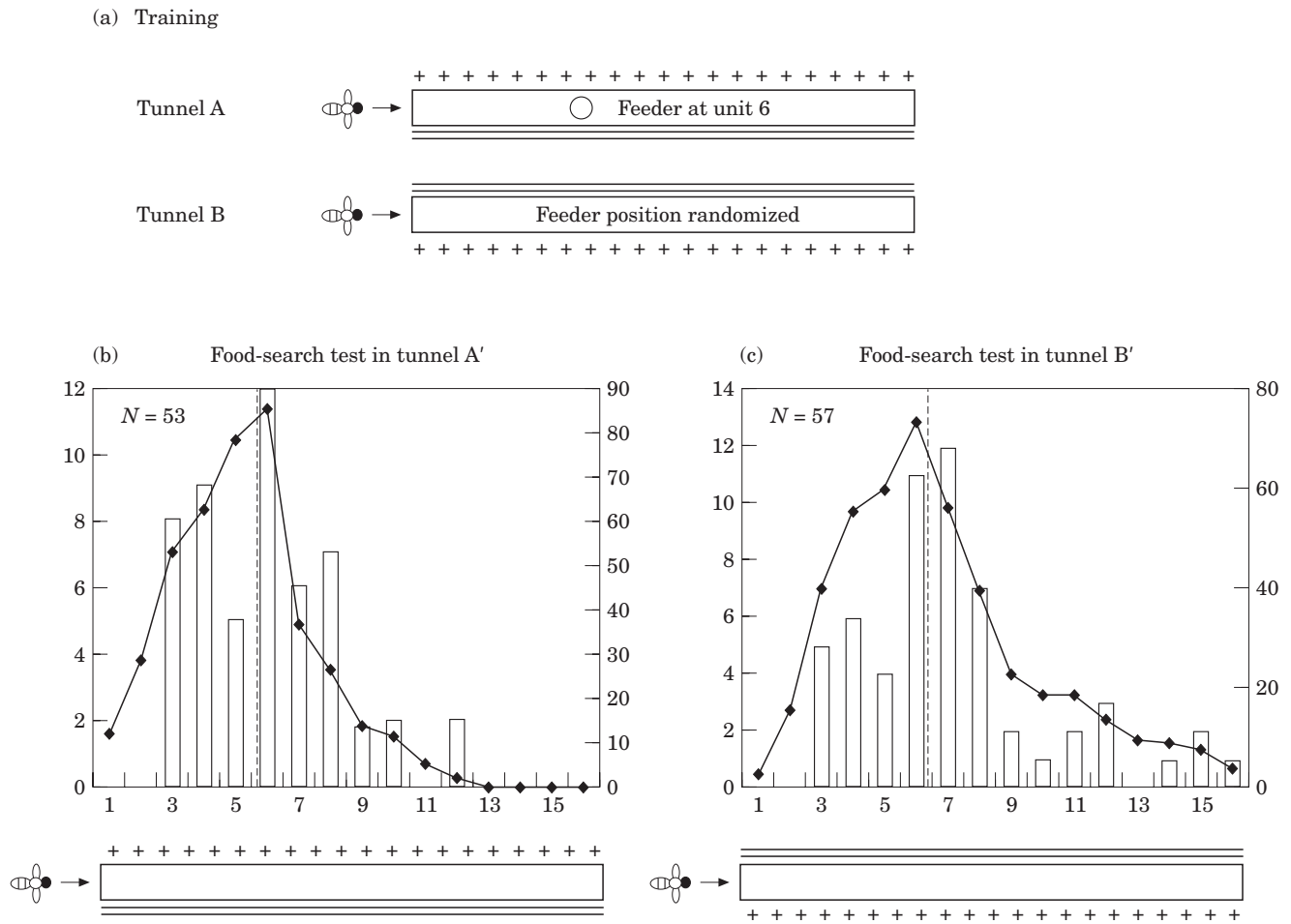


Figure 5. Experiment to investigate whether bees can transfer the reading of odometric information from one eye to another. (a) Training. Bees were trained simultaneously in two tunnels, A and B. Tunnel A carried a random texture on the left wall and axial stripes on the right wall, and offered a reward at a distance of 6 units from the tunnel entrance. Tunnel B carried a random texture on the left wall and axial stripes on the right wall. In this tunnel, the position of the reward was varied randomly from one visit to the next. The position of the reward was therefore predictable in tunnel A, but not in tunnel B. Bees trained in this way were subjected to food-search tests in tunnels A' or B' (identical to A or B). (b) When tested in tunnel A', they searched at a mean distance \pm SD of 5.56 ± 1.46 units (dashed vertical line), which was only marginally significantly different ($t_{52}=2.19$, $0.05 > P > 0.03$) from the position of the reward in tunnel A during training (at unit 6). (c) In tunnel B', the mean search position was 6.23 ± 2.19 units, which was again not significantly different ($t_{56}=0.79$, $P > 0.40$) from the position of the reward in tunnel A during training. The mean searching positions in tunnels A' and B' are not significantly different ($t_{108}=1.87$, $P > 0.05$). Other details as in Fig. 2.

Experiment 4: A Possible Role for Interoocular Flexibility

We have seen above that bees can use a 'naive' eye to measure out a distance originally learned through visual input to the other eye. Of what use is this capacity to a bee in real life? Consider a bee flying to a food source on a route parallel to the edge of a cliff or a forest. The eye facing the cliff (or forest), eye 1, would experience high image speeds, while the other eye, eye 2, would see relatively low image speeds. Therefore, odometric information would be provided primarily by eye 1. On the way back from the food source, however, each eye would experience the opposite situation, so that odometric information would now be available only to eye 2. We know, from earlier work, that bees learn the distance to a food source only on the way to it, and not on the way

back (see Introduction). However, they use information on distance learnt on the outbound journey to navigate on the way back (Srinivasan et al. 1997). For a foraging route of the kind we are considering, the bee can navigate on the way back only if she can make use of the odometric information that is now available only to the naive eye (eye 2). Does she do this?

To examine this question, we trained bees to find a feeder placed at a distance of 9 units from the entrance of a tunnel whose right wall carried a random texture and whose left wall carried axial stripes (Fig. 8a). Thus, bees experienced image motion only in the right eye on the way to the feeder, and only in the left eye on the way back. In the food-search tests, these bees searched in the vicinity of the former position of the feeder (Fig. 8b). Thus, they had obviously learnt the distance to the feeder. But even in the homing tests (Fig. 8c) they

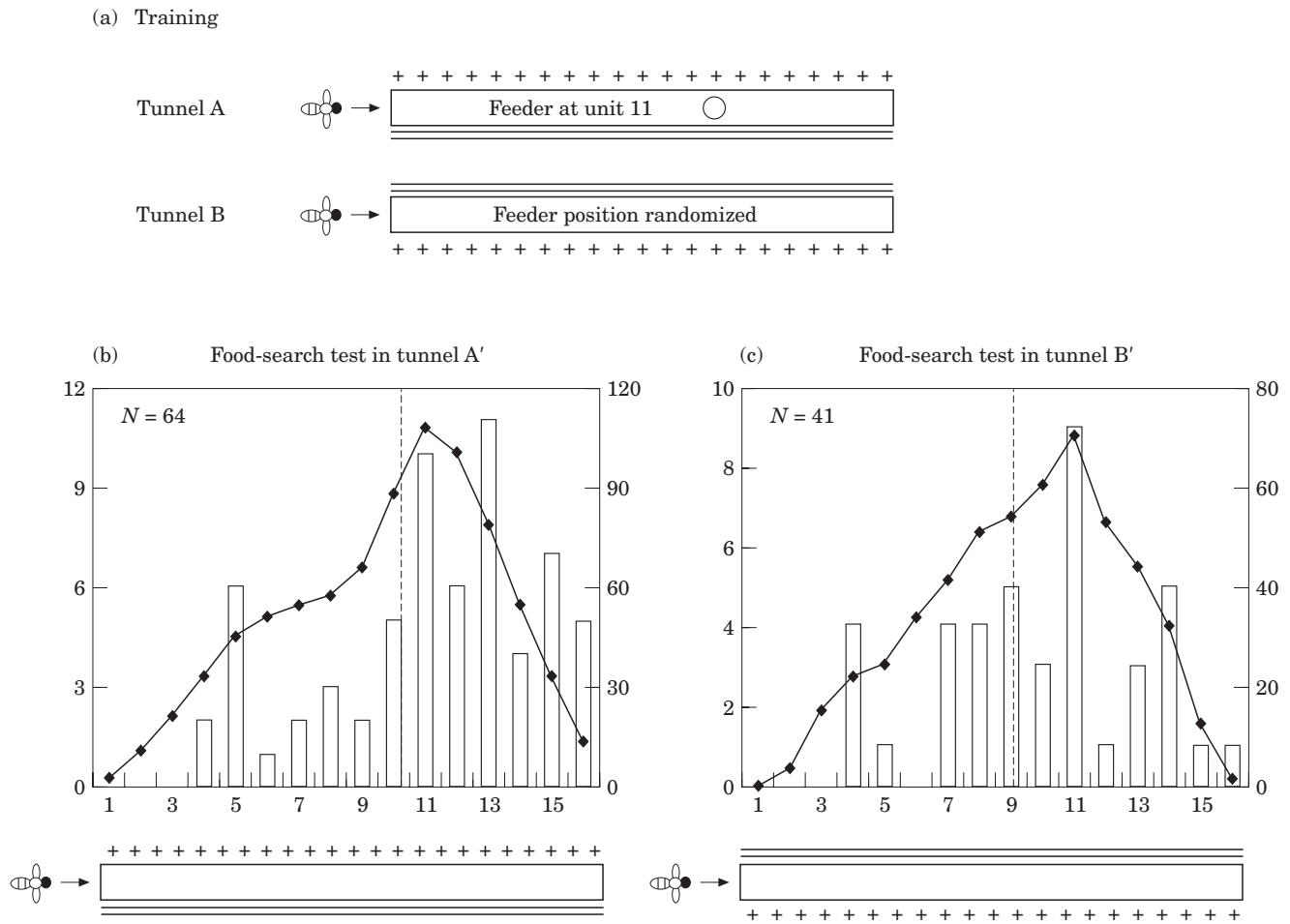


Figure 6. Experiment identical to that in Fig. 5, but with the reward placed at a different position (at unit 11) in tunnel A (a). (b) When tested in tunnel A', the bees searched for the reward at a mean distance of 10.11 ± 2.32 units (dashed vertical line), which is not far from the position of the reward in tunnel A during training, although it is significantly different ($t_{63}=3.07$, $P<0.01$). (c) In tunnel B', the mean search position \pm SD was 9.39 ± 2.53 units, which is again in the vicinity of the former position of the reward, although it is significantly different ($t_{40}=4.07$, $P<0.001$). The mean searching positions in tunnels A' and B' are not significantly different ($t_{103}=1.50$, $P>0.10$). Other details as in Fig. 2.

searched for the exit to the tunnel at the correct distance from the feeder, that is, in the vicinity of the actual position of the exit. Clearly, the bees were measuring the distance flown on the way back to monitor their progress towards the exit. Similar results were obtained in another experiment in the same tunnel, in which the bees were trained to a feeder placed closer to the entrance, at unit 4 (Fig. 9). Since we know from earlier work that bees learn distance only on the way to the food source, it follows that the bees in the experiments of Figs 8 and 9 must be comparing stored odometric information acquired by the right eye on the way in, with information acquired by the left eye on the way out.

Experiment 5: Learning Distance on Outbound versus Return Journeys

The above conclusion is critically dependent on the assumption that distances to food sources are learnt only on the outbound journey. There is good evidence for this assumption from earlier work, as we have outlined in the

Introduction. Nevertheless, we decided to test whether the assumption is valid under our present experimental conditions by carrying out one further experiment.

We trained bees to collect a reward of sugar water in a tunnel whose right wall carried a random texture and whose left wall was homogeneously white (Fig. 10a). The feeder was placed at the centre of unit 4. After a bee had alighted at the feeder and started to collect the reward, we increased the effective length of the tunnel by adding an extension, 5 units long and similarly patterned (Fig. 10a). In this training, therefore, bees had to fly a distance of 4 units into the tunnel to reach the reward, but a distance of 9 units to leave the tunnel on the way back to the hive. Furthermore, bees entering the tunnel could measure distance flown only on the basis of image motion experienced by the right eye, whereas bees leaving the tunnel could measure distance flown only through image motion in the left eye. We used only four bees, to ensure that only one bee was in the tunnel at any given time during the training, thus allowing the extension to be moved in or out as appropriate.

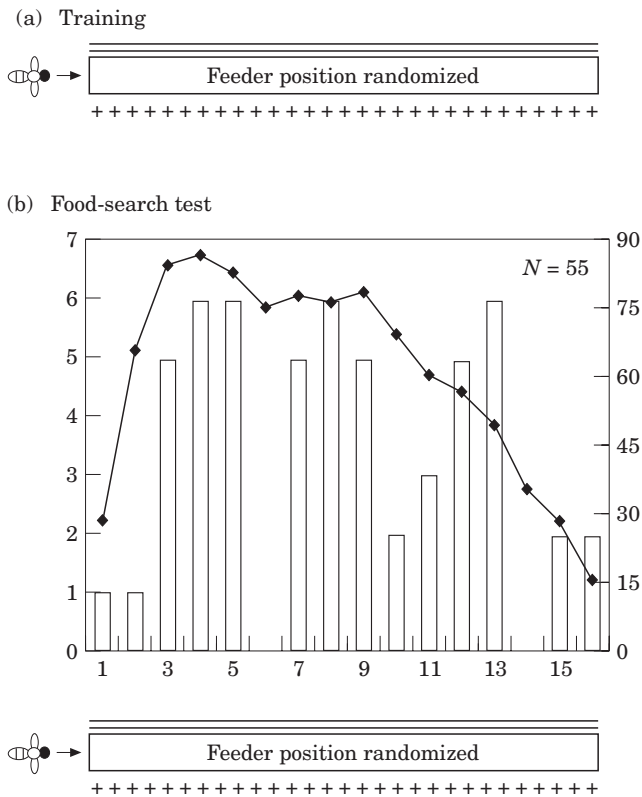


Figure 7. (a) Control experiment in which bees were trained in a single tunnel, as in tunnel B of the experiments shown in Figs 5 and 6, with the position of the reward varied randomly from one visit to the other. (b) When these bees were subjected to food-search tests in a tunnel identical to B, they showed no preferred searching location. The standard deviation of the search positions in this test was significantly larger than those in the tests in tunnel B' in the experiments described in Figs 5 and 6. F tests for variance give the following results. For comparison with the data in Fig. 5c, $F=2.21$ ($>1.90=F_{0.05}(57,54)$, $P<0.01$); for comparison with the data in Fig. 6c, $F=1.66$ ($>1.65=F_{0.05}(57,40)$), $P<0.05$.

In food-search tests, the trained bees searched in the vicinity of the former position of the feeder (unit 4, Fig. 10b). Thus, they had obviously learnt the distance to the feeder on the way in. In homing tests (i.e. upon leaving the tunnel after collecting a reward), the same bees searched for the exit at a position that was, again, approximately 4 units away from the feeder (Fig. 10c). In other words, the trained bees had not learnt the distance to the exit on the way out; rather, they were using the distance to the feeder that they had learnt on the way in, to estimate the position of the exit on the way out. Furthermore, since only one wall of the tunnel was textured, the trained bees were using the image motion experienced by one eye to estimate the distance to the reward on the way in, and motion experienced by the other eye to measure out an equal distance on the way out.

It is worth pointing out that, although bees were able to use the two eyes to memorize two different distances simultaneously when these distances represented outbound routes to two different feeders (as in the experiment

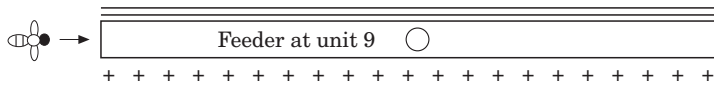
described in Fig. 4), they did not use the two eyes to learn different distances when one represented the distance to a feeder on the outbound journey, and the other the distance to the exit on the return journey (as in the experiment described in Fig. 10). This finding corroborates the conclusion that information on distance flown is learnt only on the outbound route.

DISCUSSION

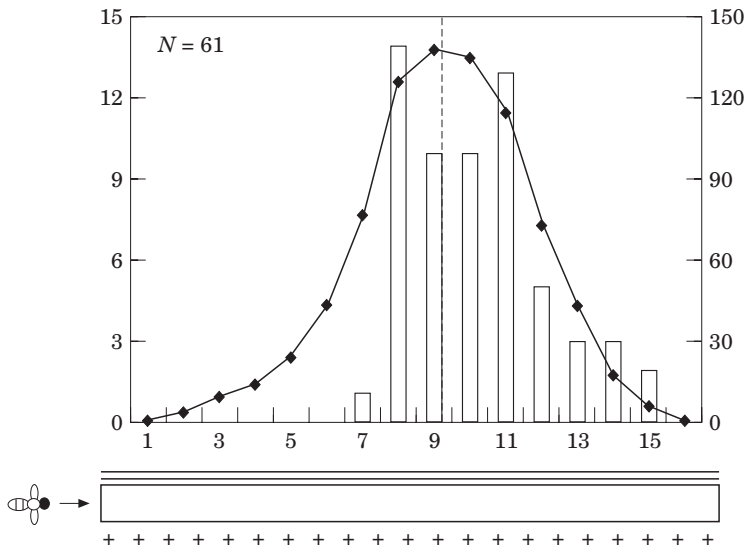
In our experimental set-up, when, say, the right-hand wall of the tunnel carried a random texture and the left-hand wall was homogeneously white (or carried axial stripes), then the bee experienced image motion primarily in the right eye when she flew towards the food source, and primarily in the left eye when she flew back to return to the hive. We say 'primarily' and not 'exclusively', because the pattern is likely to be partly visible to the other eye as well. In the bee, as in most insects, the visual fields of the two eyes overlap to some extent in the dorsal, ventral and frontal regions (Seidl & Kaiser 1981). Ideally, we would have liked to occlude one eye by painting it over, but it is difficult to get bees to continue to visit the apparatus after such treatments. Moreover, it is very difficult, experimentally, to transfer the occlusion from one eye to the other repeatedly, as would have been necessary in the experiments described in Figs 4–6. We believe, however, that the interocular overlap in the ventral region should not affect our conclusions because (1) the floor of the tunnel, being homogeneously white, did not provide any image motion cues; and (2) earlier experiments in similar tunnels have established that image motion in the ventral field of view does not play a significant role in odometry (Srinivasan et al. 1997). The overlap in the dorsal eye region is unlikely to be important, because (1) this region is specialized to analyse the patterns of polarized light in the sky (e.g. Labhart 1980) and not spatial parameters or image motion, and (2) in our experiments, bees tended to fly mainly in the upper half of the tunnel, so that the dorsal eye region did not view the pattern on the wall, but rather only the homogeneously white screen above the tunnel. The overlap in the frontal eye region, however, may be important as this means that a texture on, say, the right-hand wall, whilst providing a strong, front-to-back movement stimulus to the right eye, can also create a weak, back-to-front movement stimulation in the left eye. Some contralateral stimulation can occur even if there is no overlap in the frontal visual fields, since bees do not always fly with their long axes parallel to the tunnel axis. If there were excessive binocular stimulation, however, it would have been impossible to train the bees to learn to fly different distances based upon which wall of the tunnel provided motion stimulation, as we have done in the experiment described in Fig. 4. We must conclude, therefore, that in our experiments, it was mainly the visual input to the eye directed at the randomly textured wall that was responsible for the bee's distance preferences.

There is, however, another, quite different and probably more important role that the contralateral eye may play in the performance investigated here. This role is

(a) Training



(b) Food-search test



(c) Homing test

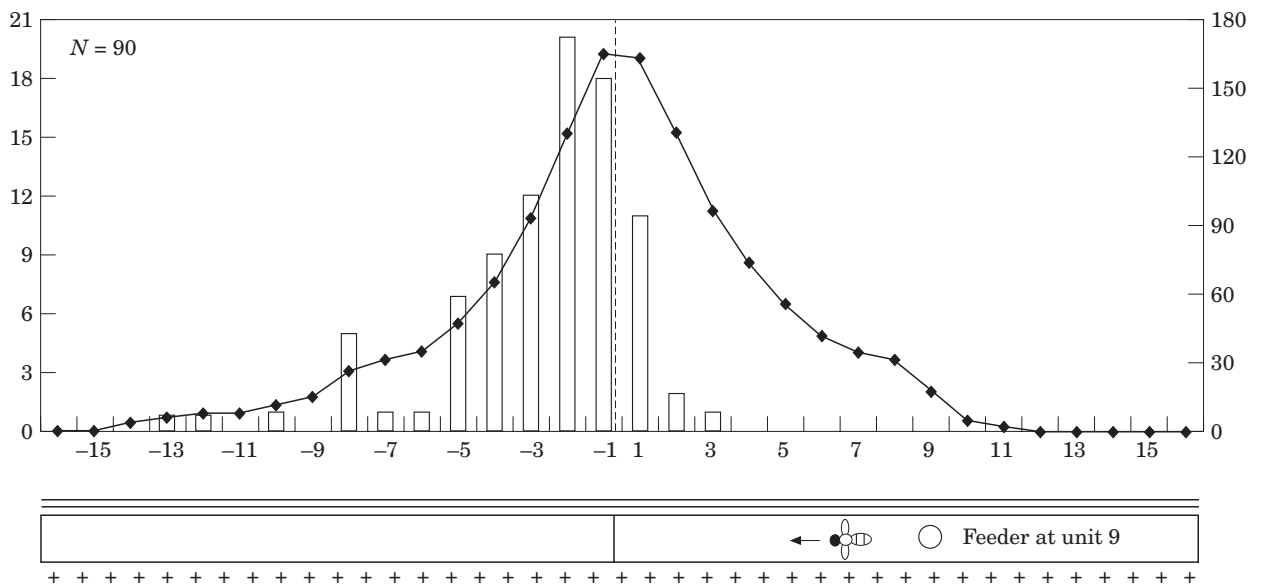
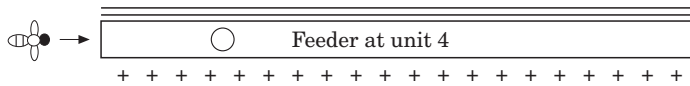


Figure 8. Experiment to investigate whether bees learn the distance from the tunnel entrance to the reward on the way in, as well as from the reward to the exit on the way back. (a) Bees were trained in a tunnel carrying a random texture on the right wall and axial stripes on the left wall, with the reward positioned at a distance of 9 units from the tunnel entrance. (b) In food-search tests they searched at a mean location \pm SD of 9.26 ± 1.0 units (dashed vertical line), which was not significantly different from the position of the reward during training ($t_{60}=2.00$, $P>0.05$). (c) In homing tests, they searched for the tunnel exit at a mean position \pm SD of -0.27 ± 1.94 units, which was not significantly different from the position of the entrance (and exit) during training ($t_{89}=1.32$, $P>0.10$). Other details as in Fig. 2.

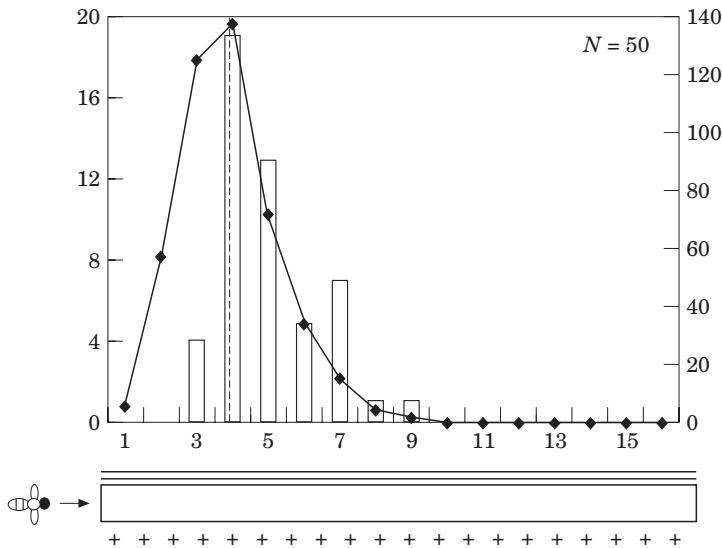
apparent from the way in which a bee entering the tunnel flew towards the expected location of the feeder. Novice bees often turned back after having flown some distance into the tunnel, returned briefly towards the exit, and

then turned back again to fly towards the feeder. Obviously, the stimuli experienced by the two eyes were transposed when a bee flew (temporarily) back towards the entrance. But this looping behaviour was rarely

(a) Training



(b) Food-search test



(c) Homing test

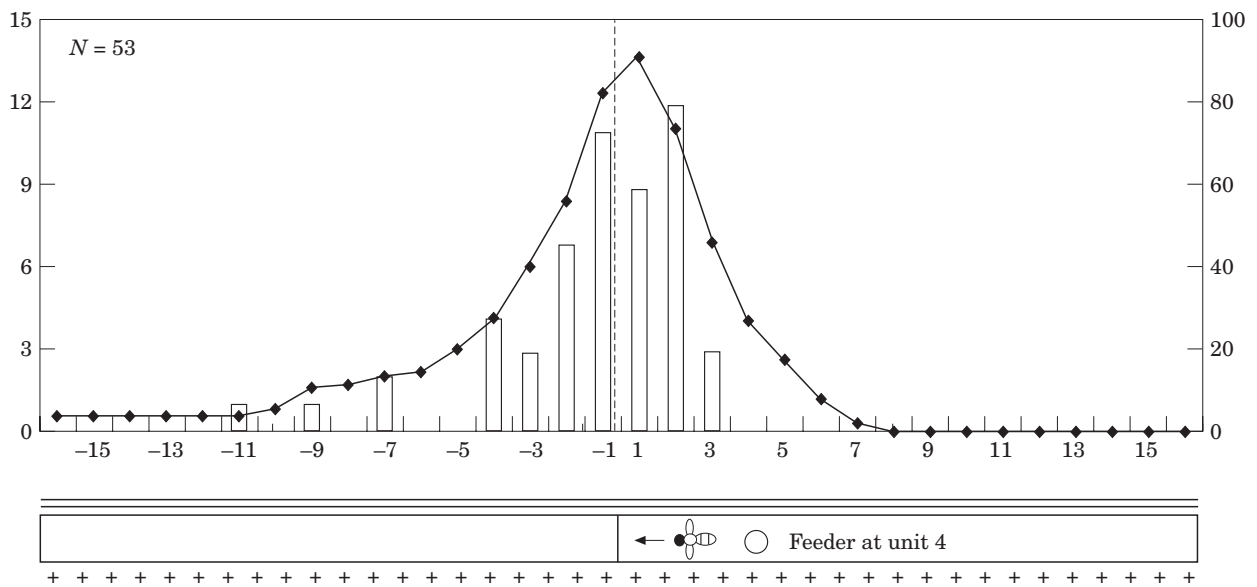


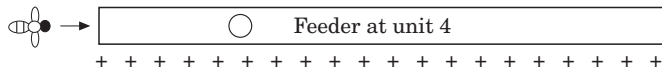
Figure 9. Experiment identical to that shown in Fig. 8, but with the reward positioned at a distance of 4 units from the tunnel entrance (a). (b) In food-search tests, bees searched at a mean location \pm SD of 3.70 ± 0.69 units (dashed vertical line), which was close to the position of the reward during training, although it was significantly different ($t_{49}=3.07$, $P<0.01$). In homing tests, they searched for the tunnel exit at a mean position \pm SD of -0.025 ± 1.77 units, which was not significantly different from the position of the entrance (and exit) during training ($t_{52}=0.10$, $P>0.90$). Other details as in Fig. 2.

displayed by trained bees that had made more than 20 rewarded visits. The histograms in Figs 2–10 substantiate this claim: they show the spatial distributions of the first U-turns made by the trained bees when they searched for the missing feeder (or the exit). These histograms indicate

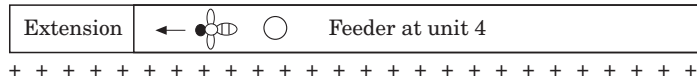
that trained bees rarely made premature U-turns. In other words, when a trained bee flew in a test tunnel with a random texture on the right-hand wall and axial stripes on the left-hand wall, she experienced image motion mainly in the right eye until she reached the expected

(a) Training

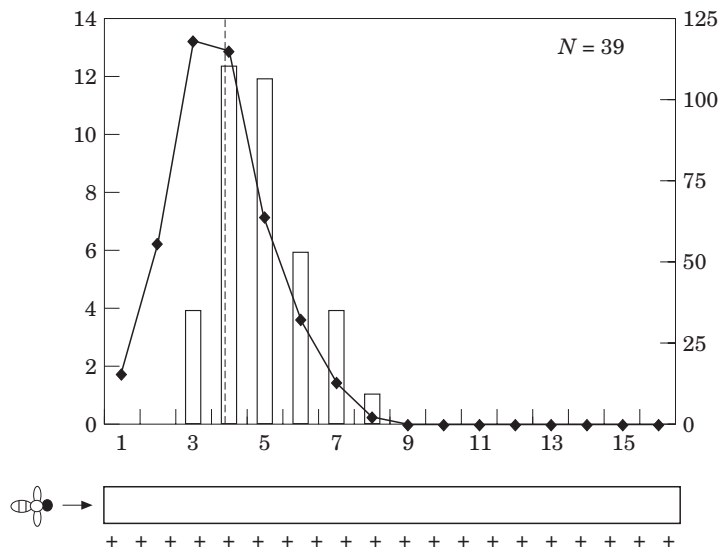
On the way in



On the way out



(b) Food-search test



(c) Homing test

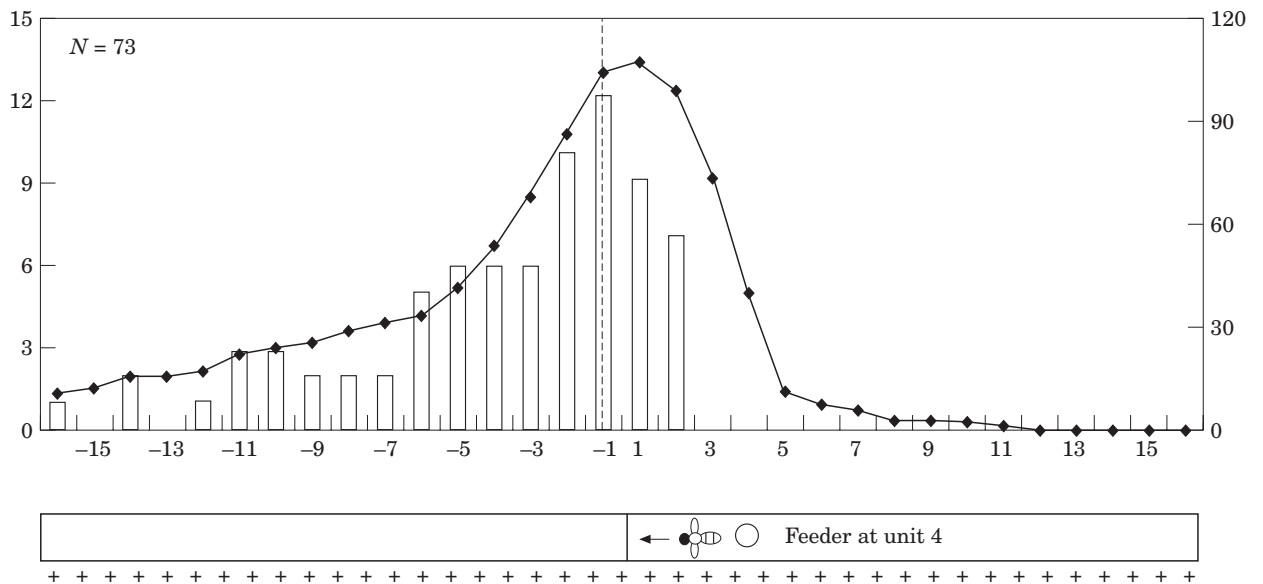


Figure 10. Experiment to investigate whether bees can simultaneously learn two distances, one from the tunnel entrance to the feeder on the way in, and another from the feeder to the tunnel exit on the way out, when these distances are different and signalled by different eyes. (a) The tunnel carried a random texture on the right wall and was homogeneously white on the left wall. Bees entering the tunnel had to fly a distance of 4 units to reach the reward, but bees leaving the tunnel after collecting the reward had to fly a distance of 9 units to reach the exit, because of the 5-unit-long extension that was added to the tunnel whilst they were feeding. In the food-search tests (b), bees trained in this way searched for the feeder at a mean distance \pm SD of 3.71 ± 0.60 units from the tunnel entrance (dashed vertical line), which was close to the position of the reward during training although it was significantly different ($t_{38}=3.02$, $P<0.01$). In homing tests (c), they searched for the tunnel exit at a mean position \pm SD of -1.30 ± 3.85 units, which was in the vicinity of the position of the entrance on the way in (0 units), but significantly different from the position of the exit on the way out (-5 units; $t_{72}=8.21$, $P<0.001$). Other details as in Fig. 2.

location of the goal and made her first U-turn. After she had made the U-turn, and flew towards the entrance, it was the left eye that experienced motion. The situation reversed when she made the next U-turn, and so on. However, the position of the first U-turn is the one that is directly related to the bee's odometric signal: she made this U-turn when she realized that she had 'overshot' the expected location of the goal. Thus, the relevant odometric signal would be provided primarily by the right eye. Even if the bee made a premature U-turn before she arrived at the location of the reward, the temporary stimulation of the left eye (when the bee was flying towards the entrance) would, presumably, simply cause the odometric signal to decrease (to be reverse-integrated) until she made the next U-turn and resumed her flight towards the reward. Thus, if the right wall of the tunnel carried a random texture and the left wall axial stripes, then image motion in the right eye would have provided a 'positive' odometric signal when the bee was flying towards the reward, whereas image motion in the left eye would have provided a 'negative' odometric signal when the bee was flying away. The opposite would be true if the left wall carried the random texture and the right wall the axial stripes. Therefore, even if both eyes participate in generating the net odometric signal, the signal from each eye would play a different role in generating this signal, and the roles of the two eyes would be transposed when the patterns on the walls are swapped.

Our experiments reveal that bees can learn to fly to a food source at a given distance even when the odometric information (i.e. image motion) is restricted primarily to one eye. The experiments described in Figs 2 and 3, which specifically compared performance in monocular and binocular conditions, revealed that performance was only mildly impaired when one eye was deprived of image motion. This is reassuring, since the image motion that bees experience in a natural outdoor environment is rarely symmetrical with respect to the two eyes.

The experiment described in Fig. 4 shows, in addition, that bees can be trained to fly a specific distance to collect a reward when they enter a tunnel that provides motion information primarily to the left eye, and to fly a different distance when they enter a tunnel that provides motion information primarily to the right eye. In other words, they can simultaneously learn to fly different distances by using odometric signals from different eyes. Collett et al. (1996) reported that bees can be trained to fly different distances into a chamber depending upon the visual stimulus that they encounter at the chamber's entrance. In their experiments, bees learned to fly one distance when they saw a grating oriented at $+45^\circ$, and another distance when they saw a grating oriented at -45° . Our bees also encountered different visual stimuli in the two tunnels that were used in the experiments: upon entering one tunnel they saw a random texture on the right wall and axial stripes on the left wall; on entering the other tunnel they saw the opposite configuration. Evidently, the context in which a bee finds herself can trigger retrieval of the appropriate odometric distance: a useful ability when a bee forages, say, at more than one patch. The bees' ability to recall the appropriate distance for

each tunnel was not 100% accurate, however: there was some 'crosstalk'. Although the search distribution in the test in tunnel A' showed a clear peak at 9 units, there was a shoulder in the vicinity of 4 units. This shoulder was not evident when bees were trained to find the feeder at a single, fixed distance of 9 units (compare with the results of the food-search test in tunnel B' in Fig. 2). Similarly, the search distribution for the test in tunnel B', although showing a peak in the vicinity of 4 units, carried a tail that extended beyond 9 units. This tail was not evident when bees were trained to find the feeder at a single, fixed distance of 4 units (compare with the results of the food-search tests in Figs 9 and 10). It remains to be determined whether the bees in our experiments were learning to associate the distance to be flown in each tunnel with the geometrical configuration of the patterns that they encountered, or, rather, with the patterns of image motion that they experienced while flying through it (image motion primarily in the right eye in one tunnel, and primarily in the left eye in the other). Irrespective of the answer to this question, the experiment described in Fig. 4 indicates that bees can learn to measure out one distance by using image motion provided primarily by one eye, and another distance by using image motion provided primarily by the other eye.

The experiments described in Figs 5 and 6 showed that bees can learn information on the distance to a food source based on the visual motion experienced primarily by one eye, and then measure out the same distance when they are required to use the other (naive) eye. We suggest that this ability is useful when the two eyes experience different amounts of image motion on the way to a food source. Bees seem to learn the distance to a food source primarily on the way to it, and not on the way back (see Introduction). Therefore, if only the right eye, say, experiences strong image motion on the way to a food source, then the bees must be able to compare the stored information obtained from the right eye on the outbound journey with that obtained from the left eye on the homeward journey, in order to monitor progress on the way back. The experiments described in Figs 8–10 showed that the bees were indeed doing this in the tunnels.

In principle, we see two ways by which the bee's nervous system could tackle the problem of odometry on the outbound and return flights. One possibility would be to learn separately the amount of image motion experienced by each eye on the way to the food source, and to monitor progress on the way back by transposing the amounts of image motion that are expected in the two eyes. Another possibility would be to sum the image motion signals from the two eyes on the way to the food source, and to keep track of progress on the return journey by again monitoring the sum of the signals from the two eyes. The first scheme would require storage of two quantities in memory, corresponding to the image motions experienced by the left and right eyes. The second scheme would entail storing only one quantity, namely that representing the sum of the image motions experienced by the two eyes. Our results so far do not allow us to distinguish between these two schemes.

A comparison of the bees' performance in food-search tests and homing tests (Figs 8–10) suggests that bees are more precise in pinpointing the location of the food source on the way in, than in pinpointing the location of the exit on the way out. This is reflected by the greater standard deviations of the mean search location, as well as the wider search distributions in the homing tests (see Figs 8–10). One reason for this may be that the transfer of odometric information from one eye to the other, if this is indeed what occurs, introduces some imprecision, as seems to be the case in the experiments described in Figs 5 and 6. This cannot be the sole explanation, however, because homing tests reveal a poorer performance even when both walls of the tunnel are textured (see Figure 6 of Srinivasan et al. 1997), in which case there is no need to transfer information from one eye to the other. Other reasons for the reduced precision in the homing tests may be that (1) bees are less precise in locating landmarks than food sources; or (2) odometry is less precise on the homebound journey than on the outbound route.

In hymenopterans, interocular transfer of learning (the ability to learn a task through visual input into one eye, and to carry out this task when the same visual input is presented to the other (naive) eye) has been demonstrated in a few situations. For example, the desert ant *Cataglyphis* learns to navigate back to its nest by using visual information based on a celestial compass and on landmarks encountered on the way, and it can do so even when one eye is covered (Wehner & Müller 1985). When *Cataglyphis* is trained to 'home' with one eye occluded, and is subsequently tested by covering the trained eye and uncovering the naive one, it shows interocular transfer if the task requires use of the celestial compass, but not if the task involves the use of earthbound landmarks (Wehner & Müller 1985). Bees with one eye occluded can learn the colour of a food source using a single eye, but they are unable to recognize the same colour when it is presented to the untrained eye (V. Pelzer, unpublished data). Similarly, bees can learn to distinguish between two different sites based on colour or pattern information presented to a single eye, but they are unable to distinguish these sites when the same visual information is presented to the naive eye (Giger & Srinivasan 1997). Bees can learn to use the position of a spatial feature (such as an edge) presented to the lateral visual field of a single eye, to pin-point the location of a frontally positioned target; but they cannot locate the target when the feature is presented to the untrained eye (Lehrer 1994). However, when detection of this feature involves the use of motion cues, bees show interocular transfer of learning (Lehrer 1990, 1994). Thus, whilst hymenopterans can clearly learn to perform a variety of tasks using one eye, their ability to transfer this learning to the naive eye depends upon the nature of the task at hand.

From the results shown in Figs 5 and 6, and Figs 8–10, we might have concluded that bees indeed transfer learned odometric information from one eye to the other. However, this conclusion cannot be drawn before we know whether bees learn the amount of image motion experienced by each eye separately on the way to the

feeding site, or whether they simply sum the image motions experienced by the two eyes, as discussed above. Perhaps this question can be explored in future work by using moving visual patterns on one or on both walls of the tunnel.

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