Honeybee navigation: distance estimation in the third dimension

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Summary

Honeybees determine distance flown by gauging the extent to which the image of the environment moves in the eye as they fly towards their goal. Here we investigate how this visual odometer operates when a bee flies along paths that include a vertical component. By training bees to fly to a feeder along tunnels of various three-dimensional configurations, we find that the odometric signal depends only upon the total distance travelled along the path and is independent of its three-dimensional configuration. Hence, unlike walking desert ants, which measure the distance travelled in the horizontal plane whilst traversing undulating terrain, flying bees simply integrate the image motion that is experienced on the way to the goal, irrespective of the direction in which the image moves across the eyes. These findings raise important questions about how honeybee recruits navigate reliably to find the food sources that are advertised by scouts.

Key words: odometer, distance estimation, honeybee, *Apis mellifera*, optic flow.

Introduction

Honeybees navigate accurately and repeatedly to food sources, as well as communicate to their colony members the distance and direction in which to fly to reach them. This information is conveyed in the so-called waggle dance (von Frisch, 1967), which consists of a series of alternating left-hand and right-hand loops, interspersed by a segment in which the bee waggles her abdomen from side to side. The length of this waggle run increases with the distance flown to reach the food source, and the orientation of the waggle axis relative to gravity specifies the azimuthal direction of the food source, relative to the direction of the sun. The waggle dance also allows analysis of the bee's internal representation of space, revealing that she is able to estimate how far she has flown in her search for food.

A bee trained to fly around a building or a mountain ridge to reach a food reward signals a direction pointing directly to the food source upon her return, a direction that she has never actually flown (von Frisch, 1967). The direction of this novel shortcut is in part based on her experience of the terrain (De Marco and Menzel, 2005), but the direction and distance to the food can also be calculated using path integration, an internal process by which an animal continuously integrates courses steered and distances travelled along the route into a global vector (Collett, M. and Collett, 2000; Collett, T. S. and Collett, 2000; Wehner and Srinivasan, 2003; Wehner et al., 2002). Interestingly, bees flying a circuitous route around an obstacle do not signal the distance of the shortcut, but rather the total distance of the detour (von Frisch, 1967; De Marco and Menzel, 2005).

A relevant measurement of distance is important not only for returning to a previously discovered food site but also for communicating the location of the food source to other colony members. Bees gauge distance flown in terms of the extent to which the image of the environment moves in the eye (Esch and Burns, 1995; Esch and Burns, 1996; Si et al., 2003; Srinivasan et al., 1996; Srinivasan et al., 1997; Srinivasan et al., 2000; Esch et al., 2001). In other words, the optic flow experienced by the eye (that is, the speed of motion of the image of the environment) is integrated over time to obtain a distance measurement. The nature of this visual odometer was unravelled by training bees to fly to a feeder placed inside a short, narrow tunnel (Srinivasan et al., 2000). When bees returned to the hive from the tunnel, they indicated a highly exaggerated distance to the feeder (~200 m), despite the fact that they had only flown 6 m. This is because the total amount of image motion depends upon the distances to the various objects that are passed; the closer the objects, the larger image motion perceived by the eye. The narrow tunnels also permit close control of the flight path of the bee. So far, all tunnel-based studies of the odometer have only investigated flight in the horizontal plane (DeMarco and Menzel, 2005; Si et al., 2003; Srinivasan et al., 1996, Srinivasan et al., 1997; Srinivasan et al., 2000). In free-flight experiments, such as the detour experiments described above, it is of course impossible to control the bees' three-dimensional flight paths.

While honeybees indicate the distance and azimuthal direction in which to fly to reach the food source, they do not appear to have a means of signalling the height or elevational

angle in their dances, at least as far as current knowledge suggests (von Frisch, 1967). To our knowledge, very little is known about how honeybees estimate distance travelled when they fly three-dimensional trajectories. Earlier studies of bees feeding from elevated food sources suggest that bees use the extent of image motion produced by the ground to estimate the distance flown (Esch and Burns, 1995; Esch and Burns, 1996). However, in these studies, the image would have moved in the same direction in the eye as during level flight (front to back in the ventral field). Thus, we have yet to explore how the estimate of distance travelled is affected when the direction of image motion changes within a given eye region. In the lateral eye regions, for example, the image of the environment would move in the front-to-back direction during horizontal flight and in the downward direction during vertically upward flight.

Here we examine how bees measure distance travelled when they fly in three dimensions, by recording their dances after they are trained to fly along short, narrow tunnels arranged in various three-dimensional configurations. These experiments are supplemented by tests in which we examine the searching behaviour of bees that have been trained to find a reward at a specific position in a tunnel that is oriented vertically or horizontally. We also investigate the extent to which the orientation of the body of the flying bee is influenced by the inclination of the flight path, in order to gain a better knowledge of how the image of the environment moves past the eyes when a bee flies uphill or downhill or ascends a cliff.

Materials and methods

Analysis of dance and body orientation

Bees (*Apis mellifera* L.) from an observation hive were trained to forage from a tunnel placed outdoors, 10 m from the hive. The tunnel was 11 cm wide, 20 cm high and 6 m long. A strip of black insect screen cloth formed the roof of the tunnel, allowing the bees a view of the sky. The walls and floor of the tunnel were lined with a black-and-white checkerboard pattern of 3 cm×3 cm squares in all experiments, except one in which the first two metres were lined with axial black and white stripes, each of width 3 cm.

For each experiment, up to 30 individually marked bees were trained to fly to a feeder containing sugar solution placed 4 m or 6 m into the tunnel. The end of the tunnel was closed, so that bees could only enter or leave the tunnel through the near end. Dances performed by marked bees returning from the tunnel were filmed using a digital video camera and later analysed. In some experiments, the bees were also filmed while flying through the tunnel, by positioning a video camera to view them side-on through a 4.5 cm-diameter aperture in one of the side walls. These video images were then analysed to estimate the body orientation during various flight conditions.

Tunnel configurations

In the first series of experiments, the bees flew in a 6 m straight tunnel that was oriented either (a) horizontally, (b) on a 48° slope or (c) vertically. In (b) and (c), the bees entered from the top of the tunnel and flew downward. In a second series of experiments, the first 2 m of the tunnel was oriented vertically, and the subsequent 4 m horizontally, with the entrance at the top of the vertical section. In one experiment, the checkerboard pattern in the vertical section of the tunnel was replaced by an axial stripe pattern. This was used to create a condition in which the optic flow experienced by the bees in the first 2 m of the tunnel was very weak.

Data analysis

The walls of the observation hive were transparent, thus enabling the viewing and filming of bee dances. A dance performed by a bee returning from the tunnel typically consisted of a number of loops. Most of these loops displayed a waggle component, whereas a few did not. The duration of the waggle component and the percentage of waggle loops were measured for 50 dances under each experimental condition. The Student's *t*-test and single-factor ANOVA were used to test for statistically significant differences between the mean waggle durations under different flight conditions.

The body orientations of bees in flight were calculated from the orientation of the long axis measured through a frame-by-frame analysis of the side views from the video data. One frame per flight was analysed from 30 bees trained under each experimental condition. The Student's *t*-test was used to test for the statistical significance of any differences between the body orientations measured for flights in the horizontal, oblique and vertical tunnels.

Analysis of food searching behaviour

20 honeybees were trained to fly in a 3 m tunnel, with the same cross section and visual texture as described above, to find a reward of sugar solution placed 2 m into the tunnel. In the first experiment, a horizontal tunnel was placed 3 m above the ground on a roof. The hive entrance was 1.25 m above the ground. The horizontal distance from the hive to the entrance of the tunnel was 4 m. In a second experiment, the tunnel entrance was kept in the same position relative to the hive, but the tunnel was now oriented vertically with the bees entering from the top and flying down. The feeder was suspended 2 m down the tunnel by means of a string. During training, the position of the tunnel was frequently changed to prevent the bees from using external landmarks to gauge their position in the tunnel. (This procedure was not necessary in the first experiment, where bees viewed only the sky and no external landmarks whilst flying in the tunnel.) After training, the bees were tested one by one in an identical, fresh tunnel that carried no reward.

Data analysis

For the purpose of analysis, the test tunnel was subdivided into 30 units, each 10 cm long. In their search for food, the bees typically flew back and forth along the test tunnel, making a number of U-turns as they searched for the missing reward. This searching behaviour was quantified by recording visually the positions in the tunnel at which the bee made the first four

U-turns. By measuring the number of times the bee entered each unit during these four turns, we could estimate the spatial distribution of its search (for details, see Srinivasan et al., 1997). For each test, the mean and standard deviation (s.d.) of the search position were calculated from the mean of the positions of the four U-turns measured for each bee. Student's *t*-tests were used to test for the statistical significance of any differences between the search positions in the two experiments, as well as the difference between the expected and the experimentally measured search positions.

Results

We began by training bees to fly to a feeder placed inside a horizontal tunnel and filmed the dances of marked bees returning from the tunnel with the feeder placed at distances of 4 m and 6 m from the tunnel entrance. As expected, the waggle duration increased with distance flown, with mean waggle durations of 159 ms (424 loops) and 250 ms (617 loops) for the feeder placed at 4 and 6 m, respectively (Fig. 1). This supports earlier conclusions that honeybees gauge distance flown from the extent of the image motion that is experienced by the eye *en route* to the food source (Srinivasan et al., 2000; Esch et al., 2001; Si et al., 2003). In these earlier studies, the bees flew exclusively in horizontal tunnels. Consequently, they experienced image motion that was oriented primarily in the horizontal direction. The above experiments with the horizontal tunnel provide a baseline against which to compare data obtained from flights in tunnels of other orientations, described below.

Oblique and vertical directions of optic flow

A fresh group of bees was trained to fly down a tunnel on a 48° incline, again with the feeder placed initially at 4 m and then 6 m from the entrance. The slope of the tunnel was chosen such that a 6 m length along the tunnel corresponded to a horizontal distance of 4 m. If the bees were extracting and using just the horizontal component of the oblique direction of image motion experienced in the tunnel, we would expect the mean

waggle duration obtained with the feeder at 6 m to be similar to that obtained with the feeder placed 4 m into a horizontal tunnel. This proved not to be the case. The mean waggle duration of 261 ms (494 loops) measured for bees returning from the feeder placed 6 m down the oblique tunnel is significantly different from that obtained for a 4 m horizontal flight (*P*<0.001, *N*=16, 15) but is not significantly different from that measured for a 6 m flight in the horizontal tunnel (*P*>0.57). Correspondingly, the waggle duration of 154 ms (463 loops) after a 4 m flight in the oblique tunnel is not significantly different from that measured after a 4 m flight in the horizontal tunnel (*P*>0.75). These results indicate that the direction of image motion is not taken into consideration in gauging the distance flown. To confirm these results, a new group of bees was trained to fly initially 4 m and then 6 m down an identical, but vertically oriented, tunnel with a horizontal projection of close to 0 m. Again, the mean waggle durations of 143 ms (460 loops) and 234 ms (434 loops) were not significantly different from those obtained after feeding at corresponding distances in the horizontal tunnel (4 m, *P*>0.19; 6 m, *P*>0.16). These results, together with those from the oblique tunnel, clearly show that the orientation of the flight path is not factored into the odometric measurement (Fig. 1).

Abrupt changes in the direction of optic flow

In the experimental conditions given above, the bees experienced a more or less constant direction of image motion throughout the length of their flight tunnel. Will the direction of image motion influence the distance estimate if it is drastically changed *en route*?

To test this, we used an L-shaped tunnel consisting of a 2 m vertical section followed by a 4 m horizontal section. The bees were trained to forage at a feeder placed initially 4 m and then 6 m into the tunnel. To reach the feeder at 6 m, the bees thus had to fly 2 m down the vertical section, change flight direction abruptly at the corner and then fly 4 m along the horizontal section. If the odometer treated image motion in the vertical and horizontal directions equally, we would expect the mean waggle duration with the feeder at 6 m to be similar to that for

Fig. 1. The effect of varying tunnel length and orientation on the mean waggle duration of bees returning to the hive after feeding inside the tunnel. Values are means ± s.d. for each experimental condition. The walls and floor of the tunnel were lined with checkerboard patterns in all cases but one, where axial stripes (Ax) were used instead. The mean waggle duration varies with the length of the tunnel but not with its three-dimensional configuration. Abbreviations: 4m, 4 m tunnel; 6m, 6 m tunnel; h, horizontal tunnel; o, oblique tunnel, 48° incline; v, vertical tunnel; L, L-shaped tunnel, with a 2 m vertical section followed by a horizontal section.
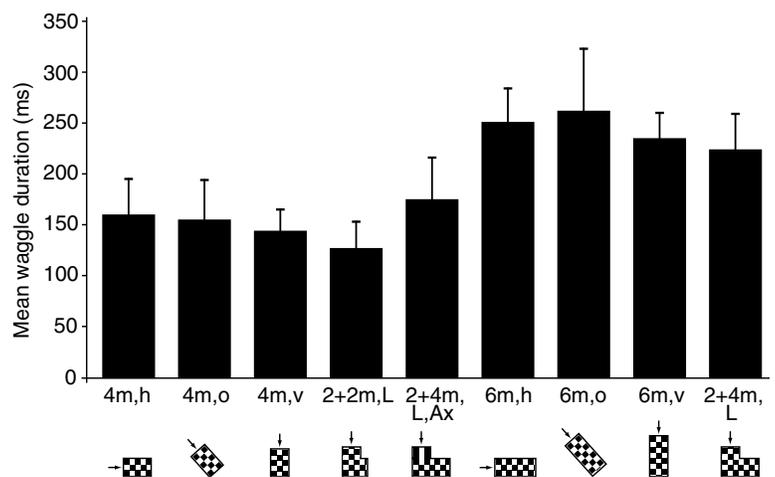
a 6 m flight in any of the other tunnel configurations described above. This is precisely what was observed. The mean waggle duration of 223 ms (644 loops) was not significantly different from those for flights to rewards 6 m into the horizontal, oblique or vertical tunnels ($P>0.80$). A 4 m flight into the L-shaped tunnel elicited dances with a mean waggle duration of 126 ms (591 loops), which was again not significantly different from that obtained after a 4 m flight into any of the other tunnels ($P>0.11$) (Fig. 1).

Is optic flow the primary cue for the visual odometer?

Is the amount of image motion experienced by the eye the primary cue to gauge the distance flown to the reward? This was tested by replacing the checkerboard pattern lining the vertical section of the L-shaped tunnel with axial stripes. A bee flying through this tunnel would now experience very little image motion in the first 2 m, since it flies in a direction parallel to the stripes. Based on optic flow cues, a 6 m flight in this tunnel would now effectively resemble a 4 m flight in a horizontal tunnel. This is also what is observed. The mean waggle duration of 174 ms (577 loops) is significantly different from that measured after a 6 m flight in the horizontal tunnel ($P<0.01$, $N=12$, 16) but corresponds well to the waggle duration after a 4 m flight in the same tunnel ($P>0.13$) (Fig. 1).

The percentage of waggle loops in the dance

In an earlier study, Si et al. documented that dances observed after a flight in a narrow tunnel are often somewhat different from those elicited after free flights in open, natural terrain (Si et al., 2003). In contrast to dances following long flights outdoors, where almost every loop contains a waggle, the bees returning from tunnels exhibit dances in which some loops do not contain a waggle phase. The percentage of waggle-bearing loops after a flight in an experimental tunnel was found to increase systematically with the length of the tunnel (Si et al., 2003). It was suggested by Si et al. that this difference in dance behaviour reflects a conflict between the odometric signal derived from optic flow in the tunnel and the 'true' distance based on the bee's knowledge of the environment external to the tunnel.

In line with earlier studies, the percentage of waggle loops in the bees' dances increased when the feeder was moved from 4 m to 6 m for each of the three differently oriented tunnels (horizontal, 22→82%; oblique, 32→60%; vertical, 45→73%) (Fig. 2), but none of the tunnel configurations systematically elicited the largest or smallest fraction of waggle loops. From this we can conclude that the percentage of waggle loops increases with increasing flight distance in the tunnels but that the different orientations of the tunnels, and consequently the different directions of optic flow, do not influence the internal conflict between the odometric signal and the 'true' distance in any predictable way.

Does the dance encode the third dimension?

Visual observation did not reveal any striking differences in the qualitative properties of the waggle dances elicited by
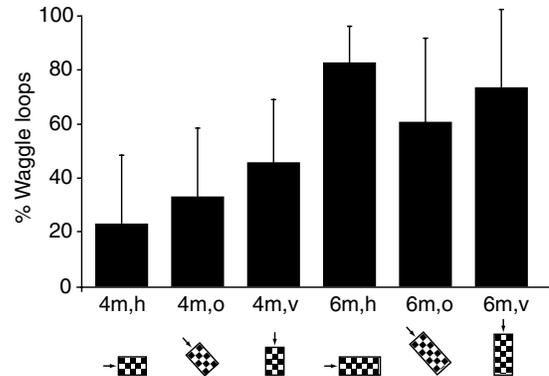

Fig. 2. The effect of varying tunnel length and orientation on the percentage of waggle loops of bees returning to the hive after feeding inside the tunnel. Values are means ± s.d. for each experimental condition. The proportion of waggle loops varies consistently with tunnel length, but not with tunnel orientation. Abbreviations: 4m, 4 m tunnel; 6m, 6 m tunnel; h, horizontal tunnel; o, oblique tunnel, 48° incline; v, vertical tunnel.

flights in the horizontal, vertical, oblique and L-shaped tunnels. This was also true for the directional component of the dance. Although we did not measure and analyse dance directions quantitatively in this study, visual observation indicated that these directions were in good agreement with the direction to the food source under all experimental conditions. Thus, it appears that there is no obvious code for signalling the height of the food source, either in terms of linear distance or angular elevation. However, a negative result such as this must always be treated with caution. For example, one cannot exclude the existence of acoustic signals in the dance that may carry information about the elevation of the food source. This possibility exists at least in stingless bees (Nieh and Roubik, 1998; Nieh at el., 2003).

Accuracy of food search in the vertical and the horizontal directions

The precision of the odometer can be estimated by measuring the accuracy with which a bee is able to pinpoint the location of a food reward to which it has been previously trained in the tunnel. Bees were trained to fly to a food reward placed 2 m into a 3 m-long tunnel, lined with a checkerboard pattern, and then tested by recording their searching behaviour in a fresh tunnel that carried no reward, as described in Materials and methods. Two experiments were performed. In one, bees were trained and subsequently tested in a horizontal tunnel; in the other, bees were trained and tested in a vertical tunnel.

The results are shown in Fig. 3. The mean search position in the horizontal tunnel (at 18.3±3.8 units) was not significantly different from that in the vertical tunnel (at 18.3±4.3 units) ($P>0.81$), and neither of these two search positions was significantly different from the position of the reward (at 19.5 units) during training (horizontal, $P>0.76$; vertical,

P>0.71). Furthermore, both searching distributions exhibit a similar width: the widths at half height are 7 units and 10 units for the horizontal and vertical tunnels, respectively. This finding lends further support to our conclusion that the ability to gauge the distance to the food is independent of the orientation of the tunnel. The slightly broader peak of the search distribution in the vertical tunnel may arise from the fact that vertically downward flight is less common than horizontal flight and from the possible reluctance of bees to enter and fly down the vertical tunnel.

Body orientation during flight

Do the bees change their body orientation in the differently oriented tunnels, so as to maintain a constant direction of image motion in the eye? This is an interesting possibility that can be easily examined. To maintain a constant direction of image motion over the eye in the horizontal and the vertical tunnels, the bee would have to dive head down into the vertical tunnel. This was never observed. The bees in the vertical tunnel flew with the long axis of the body tilted upwards at 10.1±6.5° relative to the horizontal, which differs by only ~10° from the body orientation of 19.5±8.4° measured in horizontal flight. Interestingly, whilst flying down the 48° slope in the oblique tunnel, the body was inclined at an angle of 3.3±9.3° *below* the horizontal (Fig. 4). Although the mean body orientations of bees flying in the differently oriented tunnels differ significantly from each other (*P*<0.001, *N*=26, 19, 23), these differences are not large: they never exceed a maximum of 23°.
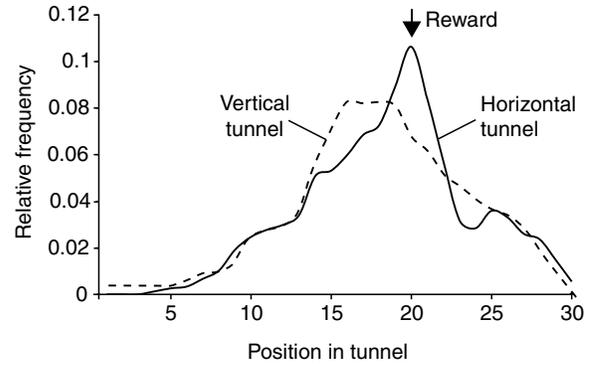
Fig. 3. The effect of tunnel orientation on the position and accuracy of food search. Honeybees were trained to find a reward at unit 20 (arrow) of a horizontal tunnel or a vertical tunnel and were subsequently tested in an identical, fresh tunnel that carried no reward. The mean searching positions in the two experiments are not significantly different from each other or from the position of the feeder during the training.

Our video films do not permit measurement of the orientation of the bee's head relative to the long axis of the body. Nevertheless, it would be safe to conclude that the bee is physically unable to tilt its head as much as would be required to experience a consistent direction of optic flow over the eye under the different experimental conditions.
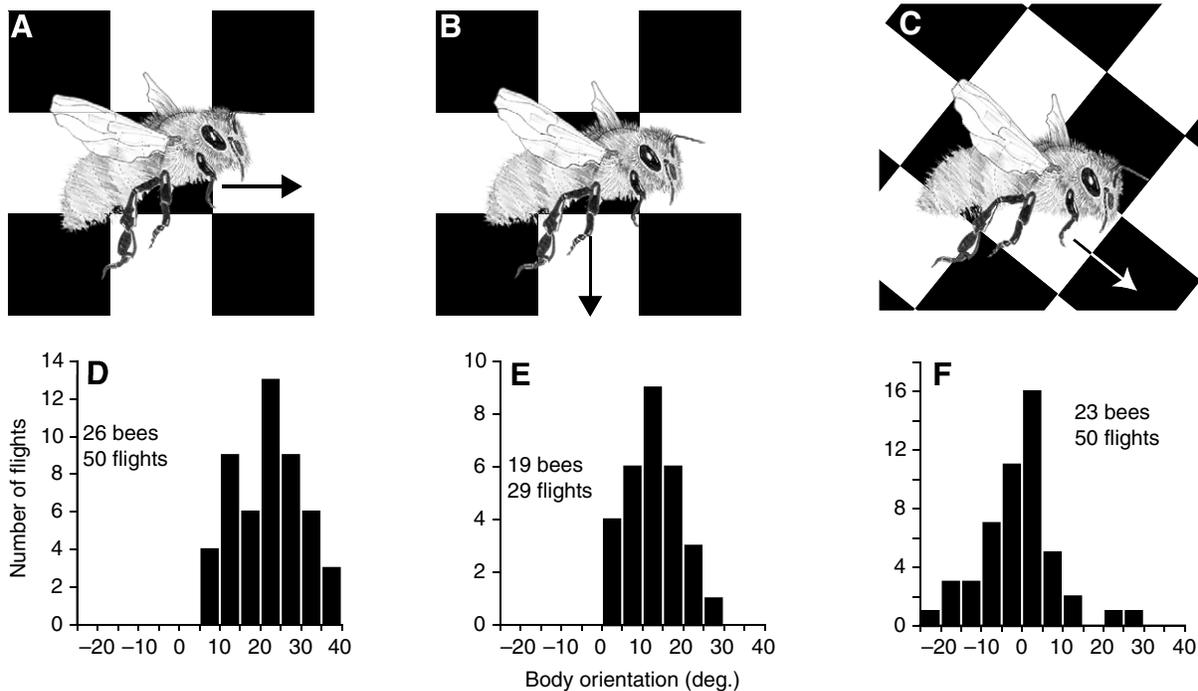
Fig. 4. The effect of the inclination of the flight path on the body orientation of a flying bee. The black-and-white squares depict the checkerboard pattern in the (A) horizontal, (B) vertical and (C) oblique tunnels. In each case, the drawing of the bee illustrates the mean body orientation of (A) 19°, (B) 10° and (C) –3°. Arrows indicate the direction of flight. Histograms show the number of flights representing each angle of body orientation, binned in 5° intervals, as measured in the (D) horizontal, (E) vertical and (F) oblique tunnels.

Discussion

The role of optic flow in distance estimation

Our findings lend further support to the notion that the primary cue used by honeybees to gauge distance travelled is optic flow integrated over time (Esch and Burns, 1995; Esch and Burns, 1996; Esch et al., 1994; Esch et al., 2001; Si et al., 2003; Srinivasan et al., 1996; Srinivasan et al., 1997; Srinivasan et al., 2000). When the checkerboard pattern lining the 2 m vertical section of a 6 m-long L-shaped tunnel is replaced by axial stripes, the distance estimate changes from 6 m to 4 m (Fig. 1). This is consistent with what one would expect on the basis of optic flow cues. Given the weak image motion cues in the 2 m vertical section, the visual odometer does not start to run until the bee enters the 4 m section lined with checkerboard pattern. The slightly longer distance estimate in the L-shaped tunnel, compared to the 4 m horizontal, vertical and oblique tunnels, is to be expected, as the weak motion cues in the axial striped section are probably sufficient to provide some drive to the sensitive odometer (Si et al., 2003). Optic flow cues could have been picked up from small imperfections in the axial pattern, from stray shadows or from the insect mesh.

Tunnel flights versus outdoor flights

The narrow tunnels used in the present study are known to magnify the perception of distance flown by a factor of 31 compared to flights in natural terrain (Srinivasan et al., 2000). A 6 m flight in the horizontal tunnel is thus equivalent to a 186 m flight outdoors. The flight in the vertical tunnel corresponds to a flight of 174 m down into the earth. This effectively means that the two tunnels simulated flights to food sources that were more than 200 m apart, but the bees conveyed roughly similar distance estimates upon their return to the hive. The 4 m horizontal projection of the 6 m oblique tunnel would translate to a horizontal flight of 124 m in natural terrain, but the bee indicated 194 m to the food source, a distance comparable to those obtained from the 6 m horizontal and vertical tunnels. It is fair to ask how these odometer readings can be biologically relevant, either to the experienced bee or to the recruit.

Odometry is scene dependent

The honeybee odometer does not run at a constant rate: rather, the rate depends upon the properties of the terrain (Barron et al., 2005; Esch et al., 2001; Tautz et al., 2004). The odometer runs faster in an environment that presents a high contrast and rich texture and runs slower in terrain with low contrast and poor texture. Consequently, equally long flights in forests and over open water will generate different distance estimates. Our findings suggest that every detour over or around objects in a terrain rich in visual texture will contribute to a longer distance estimate.

The successful use of a scene-dependent odometer relies on the fact that a honeybee follows a fixed route each time it flies to its destination (Collett, 1996). A recent study using harmonic radar (Riley et al., 2005) confirms that the recruited bees fly in the direction that the scout bee signals in her dance. Both the experienced bee and the novice will thus experience the same terrain, and therefore the same optic flow cues, on their way to the food source. This applies not only to outdoor flights in natural terrain but also to flights in short, narrow tunnels. Thus, a 'subjective' distance estimate would be just as useful as an absolute one for indicating how far a bee should fly to find the food.

Odometry and path integration

Our findings reveal that the honeybee's odometer gauges distance flown by continuously integrating the apparent motion of the visual panorama in the eye, regardless of the direction in which this motion occurs. Analysis of body orientation during flight suggests that the direction in which the image moves across the eye is very different for flights in the horizontal, oblique and vertical tunnels. Thus, at least as far as the waggle dance is concerned, bees do not signal the vector distance to the food source: rather, they signal the total length of the path that they have flown to reach it. How does this measure of total path length help a bee determine its location relative to the hive?

True path integration involves the use of a compass (to obtain estimates of instantaneous heading direction) and an odometer (to measure travel distance) (Collett, M. and Collett, 2000; Collett, T. S. and Collett, 2000; Dyer, 1998; Wehner and Srinvasan, 2003; Wehner et al., 2002). By keeping a continuous record of heading direction and distance travelled during its journey, an animal can, at least in principle, maintain a continuously updated estimate of where it is in relation to its starting point. In effect, a tortuous route is approximated by a sequence of short vectors along the path, which are summed vectorially to obtain a goal vector that indicates the distance and direction of the animal's current position relative to its starting point. We shall call this process 'vector-based path integration'.

If a bee that flies repeatedly to a food site is able to eventually navigate a direct, straight route to it, then it indicates in its waggle dance a direction that corresponds to the direction of the food source and a distance that is indicative of the optic flow that is experienced along this direct route. This is as one would expect from vector-based path integration. On the other hand, when a bee is forced to fly through a detour in the vertical plane, as in this study, it signals the correct azimuthal direction to the feeder from the hive but not the vector (shortcut) distance; rather, it indicates the total length of the path taken to the food. (With a detour in the vertical plane the azimuthal direction of the flight path remains unaffected, as one might expect.) A similar disconnection between distance and direction is observed when bees make detours in the horizontal plane (von Frisch, 1967). When a bee is forced to fly around an obstacle (such as a tall hill or building) to reach a food source (von Frisch, 1967), she indicates the true azimuthal bearing of the destination and the total length of the detour path rather than the vector (shortcut) distance. Considered together,

these findings suggest that whenever bees are forced to make a detour (either in the horizontal plane or the vertical plane), they signal in their dances (a) the azimuthal direction to the food and (b) the total path length to the food.

The observation that bees traversing a detour in the horizontal plane indicate the correct direction of the food source (the direction 'as the crow flies') implies that this direction is estimated through the use of a vector-based path-integration system – the direction of the goal vector cannot be computed correctly otherwise. On the other hand, the finding that these bees do not signal the shortcut distance (the distance as the crow flies), but rather the total path length, implies that this distance is estimated by a scalar-based path-integration system. It would appear, therefore, that, in general, bees possess two different path-integration systems that operate concurrently during the journey: a vector-based path-integration system keeps track of direction, and a scalar-based path-integration system registers the total path length. The reason for this type of representation is unclear at the moment and requires further investigation.

Specifying distance in this way may, however, be computationally advantageous in some situations. When the path to a food source is a detour, but is tightly constrained (as when flying around an obstacle in the horizontal plane, or through an L-shaped tunnel in the vertical plane), the direction signal in the dance would specify the true azimuthal direction of the goal, while the distance signal would indicate the total distance that the bee has to fly along this constrained route to reach the goal. A recruit would then simply have to use the directional signal as an indication of the general direction to the food and fly along this constrained route until its odometer has registered the appropriate path length. On the other hand, if the route to the goal is unconstrained, then the path to the food would be a straight line and the dance would correctly signal the odometric distance along this line. Thus, such a scheme would work for constrained as well as unconstrained routes.

Let us now turn specifically to flight paths that include movement in the vertical plane. If we assume that the dance contains no indication of the altitude of the food source relative to the hive (as the evidence so far seems to indicate), then all that is conveyed to the recruit is (a) the azimuthal direction of the goal (the elevational angle is unspecified) and (b) some measure of the distance to be flown along this direction, presumably along a straight line. An outdoor experiment, conducted about 40 years ago (von Frisch, 1967), suggests how information of this kind might help recruits locate an elevated food source.

Von Frisch and his student set up an observation hive on the wall of a cliff and trained the bees to a feeder suspended 53 m directly above the hive. In their dances, the bees indicated a direction directly into the cliff face. Unable to fly in this direction, the recruits flew up the wall instead, presumably facing the wall throughout the flight. The distance estimation given in the dance was unfortunately not measured in these experiments, but in the light of our findings we would expect the bees to have signalled the correct distance to reach the elevated feeder. The fact that the dancing bees were signalling a clear direction indicates that they were performing a waggle dance even when the food source was only 53 m away from the hive, probably because of the strong optic flow cues that would have been generated by flying upwards close to the wall of the cliff. A longer distance estimate, with the same directional component, would presumably have guided the recruits over the cliff to a food source on the plateau.

Esch and Burns (Esch and Burns, 1996) trained one group of bees to forage from a feeder on the ground, 158 m from the hive. A second group of bees was also trained to a feeder 158 m from the hive, but now located on top of a 50 m-high building 150 m from the hive. Both groups of bees signalled the same distance upon their return to the hive. The foragers' route was not observed during this experiment, but a path to the base of the building and then to the roof (i.e. along the route they had been trained) was put forward as a possibility to explain these results. Based on our observations of bees foraging in the L-shaped tunnel, where the distance estimates in the horizontal and vertical sections are simply summed, a flight to the base of the building and then up to the roof would imply a total path length of 200 m, which is considerably larger than the distance that was actually signalled in the dances. We therefore suggest that the bees were flying a direct, 158 m route to the feeder and reporting the optic flow that they had experienced *en route*. When flying up and towards the building, the ventral visual field would have experienced progressively weaker optic flow from the ground as the bee gained altitude, but this loss would have been compensated to some extent by the increasing optic flow in the frontal field as the wall was approached, as well as by optic flow signals that could have been provided by structures such as trees and buildings in the lateral visual fields. That bees tend to fly a straight line to elevated food sources, whenever this is possible, is also suggested by the famous balloon experiment of Esch and Burns (Esch and Burns, 1996). Their experienced bees were observed to fly a more-or-less straight path to a feeder suspended from a helium balloon 90 m above the ground and 70 m from the hive.

Our experiments reveal that when bees fly routes that involve a horizontal as well as a vertical component of movement, they do not signal the vector distance to the food source: rather, they signal the total length of the path that they have flown to reach it. The situation appears to be somewhat different, however, from the desert ant *Cataglyphis*. These ants are renowned for their ability to perform large-scale foraging excursions in rather featureless surroundings and to navigate back to their nest by using path integration (Wehner and Wehner, 1990). Ants trained to move along a series of uphill and downhill channels, and later tested on flat terrain, indicate homing distances corresponding to the distance traversed in the horizontal plane rather than the distance actually travelled along their undulating path (Wohlgemuth et al., 2001; Wohlgemuth et al., 2002). Similar results were also obtained in experiments that used more complicated three-dimensional mazes (Grah at al., 2005). In order to estimate the horizontal

distance that it traverses, the desert ant must be able to continuously measure the local slope of the terrain and factor this information into its odometric computations.

With the desert ant, it would be advantageous to measure distances in the horizontal plane because this measure preserves the relative positions of locations irrespective of the undulations of the terrain (Grah et al., 2005; Wohlgemuth et al., 2001; Wohlgemuth et al., 2002). This scheme works because the ant is earthbound and all locations of interest are located on the ground, irrespective of local height. This is not the case with a flying forager, such as the honeybee, which moves in three dimensions and where food sources can occur at arbitrary heights above the ground. Here, it would seem advantageous to specify the position of the food source as a vector in three dimensions. However, at least as far as current knowledge suggests, honeybees do not appear to have a means of signalling the third dimension (height or elevational angle) in their dances. Given this, the next best thing would be to signal some measure of the total length of the path to the food source. In the case of von Frisch's cliff experiment, described above, the indication of a definite distance and direction would drive the recruits to fly up the wall until they have experienced the optic flow that is specified by the waggle dance. Clearly, a scheme of this sort will not always work adequately. In the case of an inflorescence on the crown of a tall tree, for example, such a scheme would work only if bees have some standard 'rules' by which they negotiate environments and obstacles of various kinds. For example, if foraging for flowers in a forest canopy requires, as a standard procedure, that bees first fly vertically upwards to a height above the canopy and then horizontally in the appropriate direction under the open sky, then a dance that conveys directional information and total optic flow along such a path would enable the recruit to find the goal.

There is evidence that stingless bees (*Meliponae*) are able to communicate the three-dimensional position of a food source to their nestmates (Nieh and Roubik, 1998; Nieh et al., 2003). The mechanisms by which they do this are not yet clear, although it is suspected that acoustic signals may be involved (Nieh and Roubik, 1998). While visual inspection of honeybee dances has yielded no evidence so far of any signalling of the third dimension, it would be of interest to analyse their dances acoustically for the possible existence of such information. Pheromone trails, if present along such routes, could ensure that the same paths are followed by the scout forager and the recruits, thus making the distance signals useful. In this context, it would be of interest to investigate where the recruits from the bees trained in the 6 m vertical tunnel search for the advertised food source and to examine, in particular, whether they search outdoors at a horizontal distance of 174 m from the hive or whether they fly vertically down the tunnel and search at a location 6 m below the tunnel entrance.

Neural substrates for visual odometry

A major challenge is to uncover the neural basis of the honeybee's visual odometer. Our finding that the image motion that is experienced by the eye is incorporated into the odometric calculation irrespective of the direction in which this motion occurs is reminiscent of the way in which peering locusts use cues based on non-directional image motion to estimate the distance to a target onto which they subsequently leap (Sobel, 1990) or flying honeybees use such cues to avoid obstacles or negotiate narrow gaps (Srinivasan et al., 1993; Srinivasan and Zhang, 1997). In neural terms, non-directional motion detection could be performed by appropriately processing the responses of the large-field, directionally selective motion-detecting neurons in the lobula plate/lobula region of the insect visual pathway (e.g. Hausen, 1993). Summing the responses of four broadly tuned motion detectors with leftward, rightward, upward and downward preferred directions would yield a non-directional, motion-sensitive response. Alternatively, the summation could be performed at the level of small-field directional motion detectors, as appears to be the case with the retinotopic, non-directional T4 cells in the lobula plate of the fly (Douglass and Strausfeld, 1996).

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