Bees can Combine Range and Visual Angle to Estimate Absolute Size

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Phil. Trans. R. Soc. Lond. B 1992 **337**, 49-57
doi: 10.1098/rstb.1992.0082

Bees can combine range and visual angle to estimate absolute size

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SUMMARY

Previous work has shown that bees can discriminate objects viewed on a vertical plane on the basis of angular size, as well as objects on a horizontal plane on the basis of range. In the present study, we first demonstrate the bees' ability to measure range to a vertical surface, and discriminate angular subtense to objects on a horizontal plane. The question whether they can combine the independent measurements of angular size and range to infer the absolute size of an object is then examined for the horizontal and vertical planes.

Bees were trained to expect a reward of sugar solution when they correctly discriminate a black circular target of fixed absolute size from a similar target which is of different absolute size. Apart from absolute size, the two targets may differ from each other in either angular size, or range, or both, depending on the experiment.

In the experiments conducted on a vertical plane, the two targets were each placed in one arm of an Y-shaped choice box. In the experiments on the horizontal plane the bees could freely fly above the targets. In both types of experiments, using a variety of test situations, the bees discriminate the target of a given absolute size irrespective of angular size or range.

1. INTRODUCTION

The idea that the arthropod visual system is able to estimate the angular size of objects that move relative to the eye is well established in the literature and several authors have concluded that flying insects can keep station relative to familiar objects by keeping them at a constant angular size on the retina (for a review, see Wehner 1981). Thus, bees trained to a circular black disc presented on a vertical plane fixate it at a particular distance and can thus memorize the angular size it subtends at the eye (Wehner & Flatt 1977). In subsequent tests, the trained bees mistake a larger or a smaller disc for the one they have been trained to if the test disc appears at the same visual angle as during training, even if it is of a different absolute size. Bees can, in addition, localize an inconspicuous feeding place with the help of a landmark placed in its vicinity (Cartwright & Collett 1979). The trained bees search at a wrong distance when the mark presented during the training is replaced by a smaller or larger one, showing, again, that they learned its angular subtense rather than its absolute size.

In a previous paper we have shown that honeybees are able to estimate the range of a contrasting disc placed on a horizontal surface irrespective of the angle subtended by the target at the eye (Srinivasan *et al.* 1989). Bees make this estimation of the range of objects of unknown spatial frequency content by use of their own locomotory movements and apparent image speed (Lehrer *et al.* 1988; Srinivasan *et al.* 1991). The use of black discs as targets on a plain white background suggests that they can use a single edge in this task. Bees can also segment their visual world into objects on the criterion of parallax caused by self-motion (Srinivasan *et al.* 1990).

Because bees can measure both the angle of the target subtended at the eye, and the distance of the target from the eye, and since the visual angle depends on distance, it is a reasonable question which follows from previous work whether bees can measure absolute target size irrespective of range by coupling together the two measurements.

However, the bees' ability to measure visual angles was previously demonstrated in the vertical plane only, and their ability to discriminate range was tested on the horizontal plane only. To examine our question, we must first ask whether bees can measure the range of objects on a vertical plane, and visual angle on a horizontal plane. In the present study we investigate these questions before we examine the bees' ability to combine the two measurements in discriminating the absolute size of objects placed on either a vertical or a horizontal plane.

2. MATERIALS AND METHODS

Freely flying honeybees (*Apis mellifera*) were trained to enter the laboratory through a hole in the window

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Phil. Trans. R. Soc. Lond. B (1992) **337,** 49–57
Printed in Great Britain

and collect a reward of sugar water from a visual pattern presented in the experimental apparatus. The bees visited the apparatus repeatedly at short intervals, typically twice every 10 min.

The rewarded pattern, termed the positive pattern, was presented simultaneously with a second, unrewarded one, termed the negative pattern. In each experiment, three to five individually marked, experimentally naive bees were trained to the positive versus the negative pattern. Each training was followed by tests, in which the bees were again presented with two patterns (a positive one with a reward and a negative one without), between which they had to choose. The percentage of choices in favour of the positive test pattern is the measure of the bees' ability to discriminate between the two test patterns.

Two series of experiments were conducted, one with patterns presented on a vertical plane, the other with patterns on a horizontal plane. The experimental procedures employed in the preliminary investigations (see Introduction) were, in principle, similar to those employed in the investigation involving learning of absolute size described here, the only difference being the particular parameters to which the bees were being trained. The procedures and targets will be described separately in each section on Results.

(*a*) *Discrimination of absolute size on a vertical plane*

The experimental apparatus, described in greater detail in previous papers (Srinivasan & Lehrer 1988; van Hateren *et al.* 1990), was an Y-shaped tunnel (figure 1). In the central chamber of the Y the bees had to make a choice between two shapes, one in each arm of the Y, each presented in the vertical plane and located at a controlled distance from the entrance to the arm.

The shapes used were black circular discs pasted on a white background. The discs D_1–D_5, used two at a time (one in each arm of the tunnel), had diameters of 39, 51, 65, 75 and 85 mm, respectively, and could be placed at fixed distances d_1–d_5 of 108, 141, 180, 207 and 235 mm from the mean choice point, respectively. The angular subtense of each disc when viewed from the decision point depends on its diameter and its distance from that point (table 1). Each disc had a small tube (20 mm in diameter) inserted in its centre. In one of the discs, termed the positive (rewarded) disc, the tube led behind the disc to a small reward box, containing a feeder with sugar water. The tube in the negative (unrewarded) disc was closed from behind. The positive and negative discs were interchanged after each two rewards in one arm of the Y, to ensure that the bees learn the pattern rather than its location. The reward box with the feeder always moved with the positive disc.

The positive and negative disc always differed from each other in absolute size. In addition, they differed in either distance or angular size or both, depending on the experiment. The visual angle, because it depends on both size and distance, could be varied in two different ways. This gave the bees the option to learn one parameter and use it to discriminate between the two discs. In each experiment, the pair of discs used was varied randomly after each four rewarded visits (two in one arm of the tunnel, two in the other), but the parameter (or parameters) by which they differed from each other was always the same throughout the experiment.

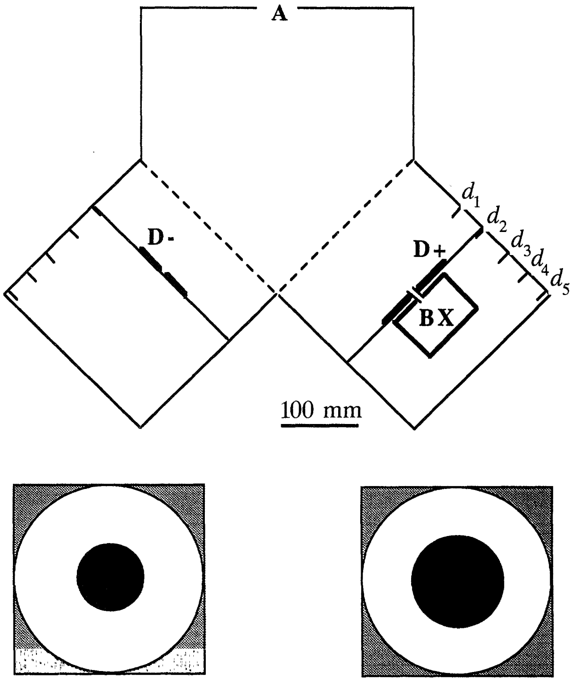

Figure 1. The Y-shaped tunnel for training and tests on a vertical surface, seen in a horizontal section. Bees enter and exit through the aperture A. Targets can be placed at different distances d_1–d_5 from the entrances to each arm (dashed lines). BX reward box containing a feeder with sugar solution. D+ positive (rewarded) disc; D− negative (unrewarded) disc. Bottom panels show a frontal view of the discs used in this example as well as the white background they are pasted on.

After an initial training period comprising at least 30 rewarded visits, testing commenced. The testing procedure was the same as the training procedure described above, the only difference being that from now on the bees decisions between the two discs were recorded. In the tests, the bees continued to be

Table 1. *Values of the angular subtenses of the discs of different diameters D_i at different distances d_j*

	disk D_i				
d_j distance	D_5 39 mm	D_4 51 mm	D_3 65 mm	D_2 75 mm	D_1 85 mm
d_1 108 mm	20.5°	26.6°	33.5°	38.3°	43.0°
d_2 141 mm	15.7°	20.5°	26.0°	29.8°	33.5°
d_3 180 mm	12.4°	16.1°	20.5°	23.5°	26.6°
d_4 207 mm	10.8°	14.0°	17.8°	20.5°	23.2°
d_5 235 mm	9.5°	12.4°	15.7°	18.1°	20.5°

rewarded whenever they chose the correct arm and found the positive disc. Their performance was assessed by recording whether they cross the entrance line of either one tunnel or the other. A correct decision scored a positive mark. Bees that enter the wrong tunnel would soon discover their error and turn round, but they get a negative mark. Only the first decision was recorded, as bees choosing the correct arm upon the first trial found the reward and made no second choice. Each test lasted for about 20 min, allowing each bee only four chances (one on each visit), each followed by a reward, twice in one arm, twice in the other. After that, another pair of discs was offered, as described above, and a new test commenced.

Apart from the tests involving discrimination between the two discs, control tests were conducted, in which the targets on the two sides were identical, at identical distances, to check that no factors other than pattern discrimination (such as side preferences or olfactory marks) offered a clue to the bees.

(*b*) ***Discrimination of absolute size on a horizontal plane***

This training procedure has also been described in detail in previous papers (Lehrer *et al.* 1988; Srinivasan *et al.* 1989). The apparatus (figure 2) consisted of two sheets of Perspex 30 cm × 40 cm, 3 mm thick, separated by a controlled distance, the lower one standing at a controlled distance above the white surface of the table (ground). The uppermost Perspex sheet acted as a barrier to control the range of the bees' flight. Typically, they would fly close to its surface while searching for the reward. The ground constituted the lowest level (the largest range), the lower Perspex sheet the middle level. The whole apparatus was enclosed in an opaque container (not shown) with a clear Perspex top within which the bees were free to fly and explore.

The targets are again circular black discs of different sizes. One disc is placed at the middle level, and another on the ground. The reward is a drop of sugar solution placed on the top sheet over the centre of the positive disc. Over the other disc a similar drop of water is placed. Two drops were needed to prevent the bees from relying on the presence of a droplet of liquid while searching for the reward.

In one set of experiments (figure 2*a*,*b*), the middle level was 60 cm below the uppermost sheet, and the ground 60 cm below the middle level. The positive disc had a diameter of 40 mm. It was placed alternately on the middle level, where it subtended a visual angle of 37°, or on the ground, where it subtended a visual angle of 18°, as viewed by the bees flying above the upper sheet (taking into account the thickness of the Perspex sheets). When the positive disc was placed at the middle level, the negative disc, placed on the ground, had a diameter of 83 mm, so that the angle (37°) it subtended at the bee's eye was identical to that of the positive disc (figure 2*a*). When the positive disc was placed on the ground, the negative one was at the middle level and had a diameter of 19 mm, subtending, again, the same visual angle (18°) as the positive disc (figure 2*b*). During training, the two situations alternated after every two or three rewarded visits.

In a second set of experiments, the middle level was

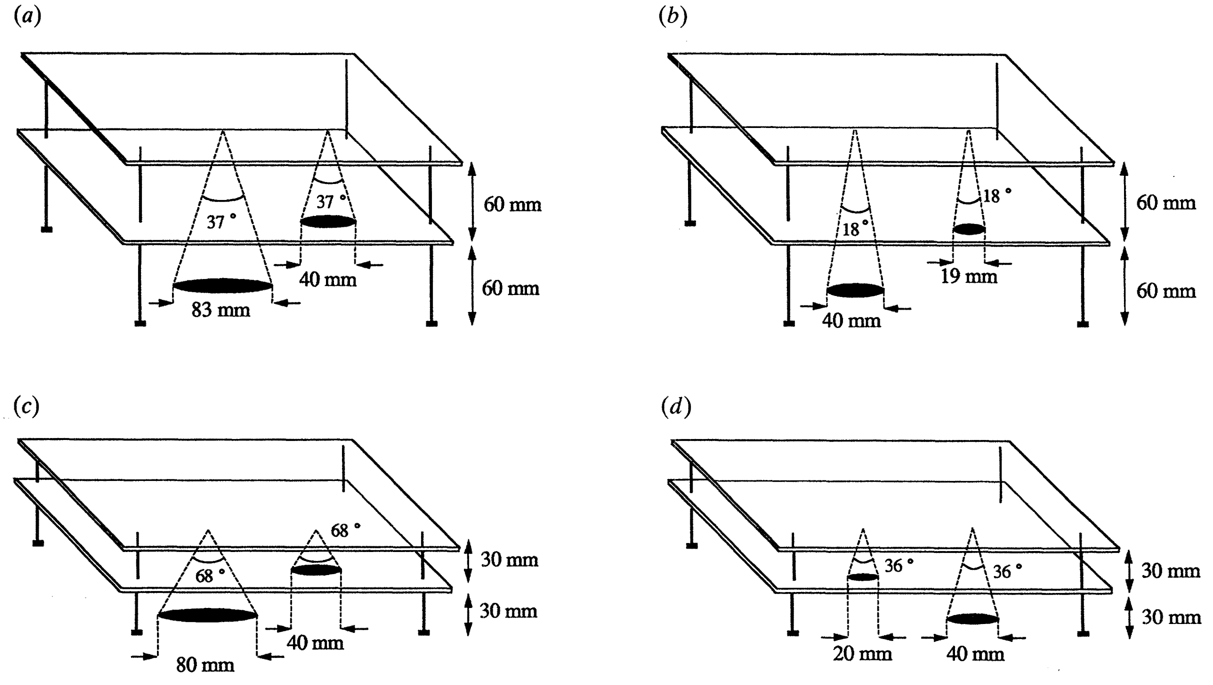

Figure 2. The apparatus for training and tests on a horizontal surface. It consists of two clean Perspex sheets placed above a white ground level with the separations shown, which differed between one experiment (*a*,*b*) and another (*c*,*d*). The targets were black discs used in pairs, placed at different locations on the ground and on the lower sheet (middle level). A drop of sugar water was placed on the uppermost sheet above one of the discs, termed positive, a drop of water above the other, termed negative. The whole of this apparatus was enclosed in a transparent box (not shown) within which the bees were free to explore.

now 30 cm above the ground and the upper 30 cm above it (figure 2*c*,*d*). The positive disc was again 40 mm in diameter, placed alternately on the ground, or on the middle level. The negative disc was 80 mm in diameter when placed on the ground, and 20 mm when placed at the middle level, so that again the positive and negative disc subtended the same visual angle, in this case 68°, when the positive disc was placed at the middle level (figure 2*c*), and 36° when it was placed on the ground (right panel in figure 2*d*). The two situations alternated during training as described above.

In either experiment, apart from the regular change of levels (and therefore of visual angle), the location of the positive and negative disc, each at its level on the Perspex sheet or on the ground, was also varied. Thus, the only cue available to the bees for recognizing the rewarded disc was its absolute size, which was the only parameter that was always kept constant, regardless of the level at which the rewarded disc was placed.

After an initial period of training, each bee's first decision on each visit was recorded, analogous to the procedure employed in the experiments on the vertical plane (see above). When the first visit was to the drop of sugar water associated with the positive disc, the bee scored a positive point, but a first visit to the drop of water scored a negative point. Sugar water does not smell and cannot be distinguished by the bees from plain water.

In addition to testing during rewarded visits as described above, tests were conducted at regular intervals in the absence of a reward. In these tests, the drop of sugar water placed above the positive disc was replaced with a drop of water, so that both discs had plain water. The bees now had to choose continually between the two discs. Each visit to the two water drops was recorded for a period of 4–5 min, after which training was resumed. Between individual tests, five to six rewards on the positive disc were offered, the bees' first choices again being recorded. This provided two different measures for the bees' discrimination performance which could later be compared with each other.

(*c*) *Analysis and statistical evaluation*

The choices of the bees between the two alternative discs were added over all tests of any given type and expressed as the percentage of choices or choice frequency, CF, in favour of the positive target. Because the bees have a choice between two targets in all tests, a value of CF $=50\%$ (or $p=0.5$) means that they cannot identify the positive target. χ^2-tests for significance against a theoretical value of $p=0.5$ were conducted whenever the value of the CF gave reason to doubt the significance. In addition, the mean value of CF obtained in all tests of any given kind and its standard deviations (s.d.) were calculated whenever a particular test was conducted at least three times and will be given in the section on Results. The s.d. is a measure of the consistency of the test results.

3. RESULTS

Experiments on a vertical surface

Learning the absolute size of a target regardless of visual angle demands that the bees establish a correlation between its size and the visual angle at which it appears from any given distance (see Introduction). Therefore, part of the bee's task in this situation is to estimate the range of the target. Range estimation irrespective of visual angle has so far been demonstrated only for targets on a horizontal surface (see Introduction). We therefore first of all conducted a preliminary investigation to see whether bees can measure the range of targets presented on a vertical surface.

(*a*) *Range measurement to a vertical surface*

Two different experiments were conducted. In one, a group of bees were trained and tested by presenting them with the positive disc at a constant range of 180 mm from the decision point (distance d_3, see table 1). Its diameter, however, was either D_1, D_3, or D_4, chosen randomly and changing after each four rewards (see Methods). The negative disc had the same absolute size as the positive disc currently in use, but was placed at a different distance (d_1 or d_5). Six different pairs of discs were used, each fulfilling these two conditions (same size but different distances). The absolute size cannot now be a clue for the bees, and they are also trained not to pay attention to the visual angle. The results (figure 3) show that the bees are perfectly able to measure range irrespective of size to a target on a vertical plane. The mean value of the

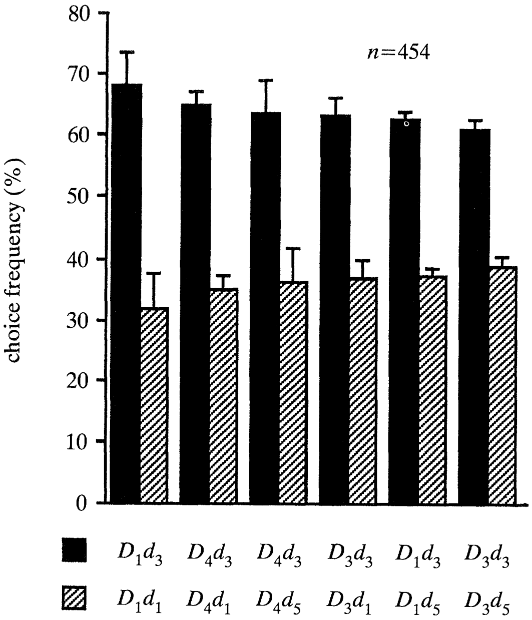

Figure 3. Discrimination of range of a target in the vertical plane. The positive disc (filled bars) was of various absolute sizes but always at the same range d_3. The negative disc (shaded bars) was the same size as the positive one but at a different range. Mean values of the choice frequencies (ordinate) are shown for each pair of test discs (abscissa). Vertical bars denote one standard deviation (s.d.); $n=$ total number of choices.

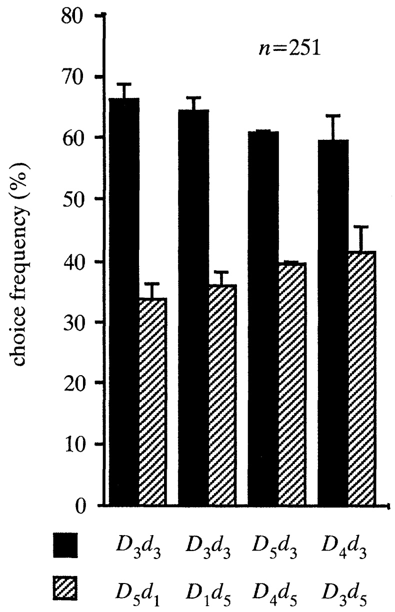

Figure 4. Discrimination of range of a target in the vertical plane. The positive disc (filled bars) was of various absolute sizes but always at the same range d_3. The negative disc (shaded bars) was at a different range but always subtended the same visual angle as the positive disc. Other details as in figure 3.

choice frequencies calculated from all of the tests conducted with all of the six pairs of patterns was CF = 64.0%, with s.d. = 3.2%.

In a control test, when two discs of the same size were placed at the training range (d_3), the result was CF = 53.8% ($n = 26$), showing that the bees have no preference for one arm of the tunnel and do not use olfactory cues which might possibly originate from the reward box.

A second group of bees were trained as before with the positive target at the fixed range of 180 mm and variable diameter (this time D_3, D_4, or D_5). The distance of the negative target again differed from that of the positive one, but its size now was chosen such that both discs subtended the same visual angle. Four pairs of discs were used, each fulfilling these two conditions (same visual angle, different distances). The visual angle cannot now be a clue for the bees and they are trained not to pay attention to absolute size. The results (figure 4) are similar to those obtained in the first experiment, the mean value of the choice frequency being CF = 62.2% (s.d. = 2.4%).

(*b*) *Training for an absolute size*

In the following experiments the bees were all trained to D_3 as the positive disc and either D_1, D_2, D_4 or D_5 as the negative one. Five series of experiments (i–v) were conducted.

(i) *Targets at different distances*

In the first series, the positive disc (diameter D_3) was placed at any of four distances d_1, d_2, d_3, or d_4 (see table 1) and therefore subtended various visual angles. The negative disc had various sizes and various distances, all chosen so that it always subtended a constant visual angle (20.5°), regardless of its size and distance. In addition, the positive and negative discs were always at different distances. The bees are therefore trained to look for a disc of constant absolute size at various ranges as compared to a disc of a different absolute size which had, in addition, a different angular size (figure 5).

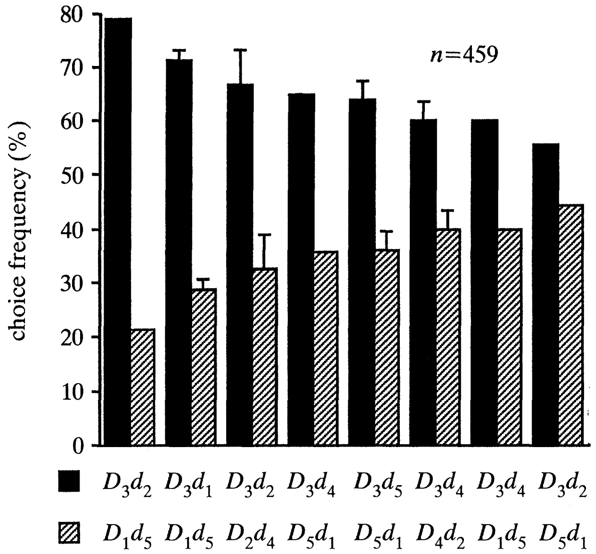

Figure 5. Discrimination of absolute size. The positive disc (filled bars) was of constant size D_3 at various distances and therefore subtended various visual angles. The negative disc (shaded bars) had a constant visual angle (20.5°) but was always at a different range than that of the positive disc. Other details as in figure 3.

The CFs obtained in favour of the positive target differ between the various tests. There is no correlation between the CFs and the differences in distance or visual angle between the two alternative test discs. However, they are always significantly higher than 50%, no matter what is the combination of targets used in the test. The mean value of all of the CFs obtained was CF = 65.7% (s.d. = 6.6%). This suggests that the bees use the absolute size of the positive disc in this discrimination task.

However, another possible interpretation of these results could be that the training was against a particular value of the angular subtense, because the negative disc always subtended 20.5° at the eye, while the positive disc did not. It is only at distance d_3 that the positive disc D_3 subtends 20.5°. This distance was never used for the positive disc in this experiment.

(ii) *Targets subtending identical angular sizes*

To exclude the above possibility, bees were trained and tested for discrimination of D_3 when the angular subtenses are the same on the two sides. Five different pairs of discs were used. In each, the positive and negative target differed in both size and distance, but subtended identical visual angles. The results (figure 6) show that the bees can discriminate the positive target from the others of equal angular subtense (CF = 64.0%, s.d. = 3.8%). This corroborates the results in figure 5 and shows that the cue the bees were using was, indeed, the absolute size of the positive disc.

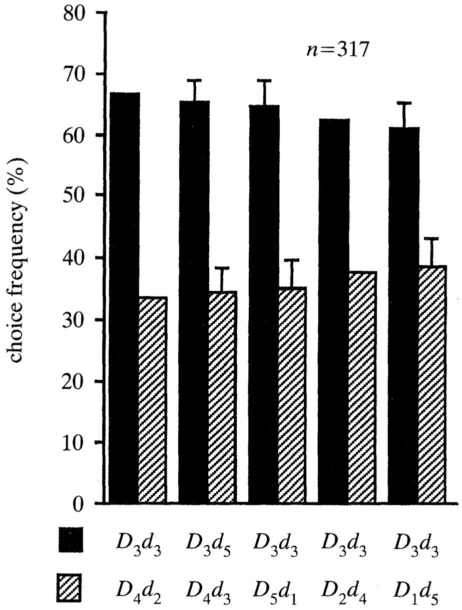

Figure 6. Discrimination of absolute size. The positive target (filled bars) was of constant size D_3, and, with one exception, also at a constant distance. The negative target (shaded bars) was at a different distance but always subtended the same visual angle as did the positive target. Other details as in figure 3.

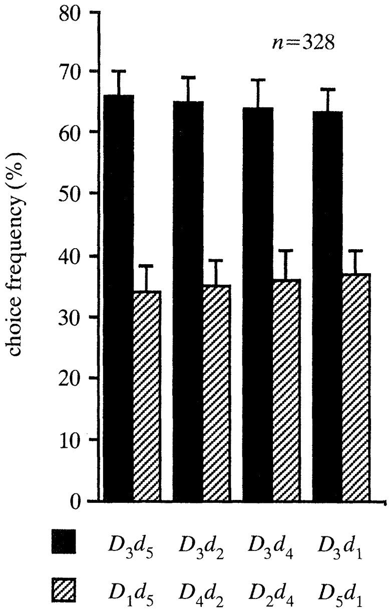

Figure 7. Discrimination of absolute size. The positive target (filled bars) was of a constant size D_3 at various distances. The negative target (shaded bars) had a different angular subtense but was always at the same range as the positive target. Other details as in figure 3.

(iii) *Targets at the same range*

In this experiment, the positive target was again D_3 placed at distances d_1, d_2, d_4, or d_5 from the choice point, as in figure 5. The negative target was D_1, D_2, D_4 or D_5 and therefore differed from the positive one in its size as well in its visual angle. However, it was always placed at the same distance as the latter. The bees were thus unable to use distance as a cue for discriminating between the two targets.

The results (figure 7) show that the bees were able to discriminate between the two discs even under these circumstances, the mean value of CF being 63.8% (s.d. = 4.2%). Because they were rewarded on D_3 placed at different distances, they must have learned to correlate each distance with the particular size of the rewarded disc, which was the only cue available to them in the task.

(iv) *Two positive targets at different distances*

As a control we set up a series of tests in which the disc of size D_3 to which the bees have been trained (see figure 7) was placed in both arms of the Y, at various combinations of distances. All the results of these tests (figure 8) demonstrate that the bees make no significant discrimination (CF = 48.5%, s.d. = 1.4%) when both discs are the same size, showing that they have not been trained to any particular range or angular subtense, and suggest, therefore, that they have been trained to look for a particular absolute size.

These tests show, in addition, that there is no preference for one arm and that no olfactory cues are involved in the bees' choice behaviour.

(v) *Tests eliminating the possible influence of the background*

In all of the experiments involving learning of size (figures 5–8), the positive disc had a constant size and, therefore, regardless of its distance, the ratio between its area and the area of the white background on which it was pasted (see Methods) was constant. Thus, apart from learning the absolute size of the disc, the bees had the option of learning this constant ratio. To examine whether or not the bees have used this cue in the discrimination task, experiments were conducted in which the background on which the discs

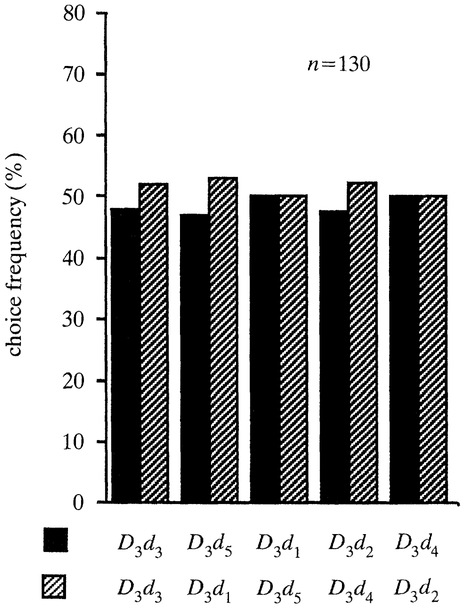

Figure 8. Results of control tests after having trained the bees as shown in figure 7. In these tests, the negative disc (shaded bars) had the same absolute size (D_3) as the positive disc (filled bars). The ranges of the discs varied from test to test. With the exception of one test, in which both discs were identical in all respects (D_3d_3 versus D_3d_3), the negative disc differed from the positive one in both range and visual angle. Other details as in figure 3.

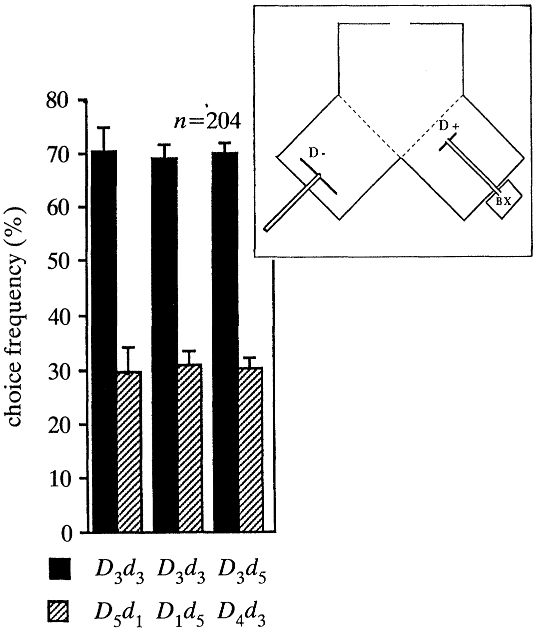

Figure 9. Results of tests without the background cue (see text for a more detailed explanation). The discs were mounted on the end of tubes rather than pasted on a white background. Here the far end of the Y-arms served as a background of constant size (inset). The ratio of disc size to background size now depended on the disc's size and range, and therefore the bees could not use it as a cue in the discrimination task. Tests were conducted with three of the pairs of discs used in the experiment shown in figure 6. Other details as in figure 3. Filled bars, positive disc; shaded bars, negative disc. Inset: scheme of the modified experimental setup used (compare with figure 1). BX = reward box.

were pasted was eliminated (inset in figure 9). The positive and negative discs were now mounted at the end of tubes whose length could be adjusted to achieve each desired distance. The reward box, which was previously hidden behind the background carrying the target (see figure 1), was now placed behind the rear wall of the arm in which the positive disc was placed. The far end of the tube penetrated that wall and ended in the reward box, and the bees were trained to walk through the tube to collect the reward. Now the rear wall of each arm, covered with light grey paper, served as background. Because its size was constant, the ratio of disc size to background size now varied depending on the size and range of the discs and could, therefore, not be used by the bees as a cue in the discrimination task.

Using D_3 as a positive target again, three experiments were conducted, using three of the five pairs of targets used in the experiments of figure 6. The results (figure 9) were similar to those of figure 6, or even slightly better (CF = 69.8%, s.d. = 2.9%), showing that the white background used in the previous experiments had no influence on the test results.

Experiments on a horizontal plane

It has already been shown that bees trained on a horizontal plane can learn the range of objects irrespective of their size (see Introduction). However, bees must also measure the visual angle subtended by the object at the eye before they can couple together angular size and range to extract information about the absolute size of the object. Discrimination of angular size has been demonstrated so far only on the vertical plane. Therefore, as mentioned in the Introduction, we first investigate the bees' ability to learn the angular subtense of targets placed on a horizontal plane.

(*a*) *Discrimination of angular size on a horizontal plane*

Using the experimental setup shown in figure 2*a*, bees were trained to a positive disc which subtended a constant visual angle of 37° at the eye. It was placed alternately on the ground, in which case its diameter was 83 mm, or on the middle level, in which case a disc of 40 mm diameter was used (see Methods). Because the range of the positive disc varied continually during the training, the bees could not rely on its range to recognize it. The negative disc had the same absolute size as the positive one, but it was placed at the alternative level and therefore subtended a different visual angle, which was either larger or smaller than that of the positive disc, depending on the level at which the discs were placed. Thus, the bees could rely on neither range nor absolute size to discriminate between the two discs. The only parameter they could use was the visual angle of the positive disc, which was always kept constant.

In the tests no reward was offered, both discs carrying each a drop of plain water (see Methods). With each of the pairs of targets used during training, the bees had to choose between the two discs, one of which was the positive one, i.e. either the 83 mm disc placed on the ground, or the 40 mm disc placed at the medium level. In each of the two situations, three tests were conducted.

The mean value of CF, calculated from all of the tests, was 69.6% (s.d. = 7.9%) (n = 429), showing that the bees learned the angular size of the positive disc regardless of range and absolute size. Even if they learned to choose the lower of two targets in one situation, and the higher one in the other, they had no cue other than the visual angle subtended by the positive disc to decide whether they are currently presented with the former or the latter.

As a control, the trained bees were given a choice between the two positive discs used during training, i.e. the 83 mm disc placed on the ground, and the 40 mm disc placed at the middle level. Here the mean choice frequency was CF = 50.9% with s.d. = 4.6% (n = 281), showing that the cue used by the bees in the discrimination task described above was indeed the angular size of the positive disc and only that.

(*b*) *Training for an absolute size*

In the following experiments the positive target had a constant size but its visual angle varied during the training. The negative target possessed the same visual angle as the positive one, but both its range and absolute size were different. The experiments were conducted using two different experimental setups (see Methods).

(i) *Experiment 1: larger ranges*

In this experiment the distance of the middle level from the bees' plane of flight was 60 mm and that of the lower or ground level 120 mm, taking into account the thickness of the Perspex sheets (see figure 2*a*,*b*). Whether the positive disc was placed at the middle or the lower level, the bees clearly discriminated it from the alternative disc placed at the alternative level, CF being 62.4% with visual angle of 37°, and 58.7% with visual angle of 18°. The mean CF obtained from all 8 tests conducted in both situations was 60.6%, s.d. = 6.7% ($n = 460$).

Counting only the first decisions during training (see Methods) rendered an even better result, CF being 71.8%, s.d. = 7.8% ($n = 227$). The differences are not surprising. We know that during tests in which a reward is absent, the obtained CFs tend to be lower than when a reward is present, because the bees shuttle between the two alternative stimuli, receiving no reward even when they choose correctly, resulting in an increased number of visits to the negative stimulus.

Because, in each of the two situations, the positive and negative discs subtended the same visual angle, the results show that the bees have learned the absolute size of the positive disc irrespective of its visual angle. Even if they have learned to prefer, in one situation, the higher disc, and in the other the lower one, they had no clue other than the absolute size of the rewarded disc to decide whether they are currently presented with the first or the second situation.

As a control, bees trained as described above were tested with two equal positive targets of 40 mm, placed at different ranges, but were unable to discriminate between them, the CF being 48.7% (s.d. = 7.9%; $n = 547$). As in the case of the targets on a vertical surface (see figure 8), this shows that the bees trained to a constant size are not trying to solve the problem on the basis of range or visual angle.

(ii) *Experiment 2: shorter ranges*

The range of the middle level was now 30 mm from the uppermost level, and that of the ground level 60 mm (see figure 2*c*,*d*), using the same positive disc as before (40 mm diameter). The negative disc again subtended the same visual angle as the positive one at each level. Again, whether the visual angle was 67.5° (when the positive disc was at the middle level) or 35.8° (when it was on the ground), the bees preferred the positive disc. The results were very similar to those obtained with the larger range (see above), the mean CF value being 58.2%, s.d. = 14.2% ($n = 697$) during tests with no rewards, and 71%, s.d. = 9.4% ($n = 519$) during rewarded visits.

Because learning the absolute size demands that the bees first learn to infer a particular constant size at a variety of different ranges, our results show that a distance of only 3 cm is sufficient for learning the range. This is in agreement with earlier results investigating the use of motion parallax in detecting raised objects (Srinivasan *et al.* 1990).

4. DISCUSSION

(*a*) *Distance estimation using various eye regions*

Previous studies have already shown that bees can estimate the range of objects placed on a horizontal plane, and therefore seen with ventral eye regions (Lehrer *et al.* 1988; Srinivasan *et al.* 1989), and of objects placed laterally, thus projecting on peripheral eye regions (Srinivasan *et al.* 1991). The present study using targets placed on a vertical plane shows for the first time that range discrimination also takes place in the frontal eye regions (figures 3 and 4). This is, of course, not surprising, given that information about the three-dimensional layout of the visual panorama can only be extracted from translational optic flow by comparing the apparent motion velocities perceived by the different eye regions.

(*b*) *Learning of unusual cues*

The present investigation involves range discrimination on a vertical plane, measurement of visual angle on a horizontal plane, and discrimination of absolute size in either plane. The same experimental setup and the same set of patterns were used in either plane to investigate two different questions. Each of the targets possessed all of the three properties we were looking at, i.e. range, angular size, and absolute size. However, to answer the different questions we had to look at them one at a time. This was achieved by varying the training procedure according to the question to be investigated. By using a pair of patterns, one rewarded, the other not, it was possible to train bees to pay attention to one particular parameter, and ignore the others. In the rewarded stimulus, the parameter to be learned – either range, visual angle, or absolute size – was always kept constant, while the others were varied at regular intervals. At the same time, the unrewarded stimulus differed from the rewarded one in just the parameter to be learned, but not in the others. Our results show that bees are able to pick up the particular parameter to which they have been trained and use it in the discrimination task when no other reliable clue is available to them in the task.

The results show that the honeybee can discriminate a target on the basis of absolute size. However, the bees do not perform very well on this task. Few of the tests rendered a value better than 70%, which is comparable to training and testing on patterns which are rather difficult to discriminate.

The possibility that the bees perform badly because they enter an artificial environment in the laboratory with items and three-dimensional structure that would be unusual in the bees' natural world can be dismissed, since they can perform very well in the same conditions if given an easier task (such as discrimination between horizontal and vertical edges, for examples, see Srinivasan & Lehrer (1988) and Van Hateren *et al.* (1990)), and anyway they appear to be undisturbed by their strange surroundings.

A more realistic explanation would be that, under natural circumstances, the bee rarely makes use of the absolute size of objects. For measuring the apparent speed of objects – a task in which the bee performs well in the context of a variety of different visually guided behaviours, including range discrimination (see review by Lehrer (1991)) – the size of objects is irrelevant. Even in typical recognition tasks, the bees need not know the size of objects. Although specialized predators could identify a particular prey, among other things, by its absolute size, as is presumably the case in the backswimmer *Notonecta* (Schwind 1978), and others might use absolute size to recognize a predator or a potential mate, we know of no object in the bee's life in which the size might be of particular importance. Landmarks are learned by memorizing their angular subtense (Cartwright & Collett 1979), or their mutual spatial relations (Anderson 1977; Gould 1987). Hive mates are recognized by their smell, and honey robbers such as bears and skunks by their dark colour and high body temperature. Finally, flowers, which are very relevant objects indeed, possess different sizes but are easily recognized by their odor, colour and shape.

Therefore, we cannot expect bees to learn the absolute size of objects, being a rather unusual cue with little significance in normal conditions, as readily as they learn other cues often used under natural circumstances. Still, the bee is able, as demonstrated in the present study, to learn even the absolute size if she has no other cue at hand, which shows how prepared the bee is to learn anything at all which might help her to identify a food source.

We thank Dr Mandyam V. Srinivasan for many fruitful discussions and much advice in the course of the experiments. One of us (M.L.) wishes to thank Professor G. Adrian Horridge and his colleagues at the Centre of Visual Science in Canberra for their kind invitation and friendly hospitality. Figure 2 was prepared by Tara Goodsell, who also helped in some of the experiments.

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Received 30 December 1991; accepted 11 February 1992