

Visual resolution of gratings by the compound eye of the bee *Apis mellifera*

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Summary

Early measurements of the resolution of horizontal *versus* vertical gratings were confirmed, with a limit near a period of 2.5° , and the resolution is similar when vertical or horizontal gratings are tested separately against grey. Bees were next trained to discriminate from a distance between gratings at 45° *versus* 135° , with no green contrast, on targets presented in a vertical plane at a fixed distance. As expected, they fail to learn; however, with green contrast but no modulation difference the resolution limit is near 3.5° . With vertical and horizontal gratings with no green contrast they discriminate but do not learn an orientation cue. In order to eliminate the orientation

cue altogether, new bees were then trained with alternating vertical and horizontal gratings *versus* grey, or with a black and white checkerboard *versus* grey. Tests of these trained bees with horizontal or with vertical gratings separately against grey again show a resolution down to a period near 2.5° . These results, taken together, show that when edge orientation alone is the cue, the limit of resolution is near 3.5° , but when receptor modulation is the cue, the limit is near 2.5° .

Key words: bee, *Apis mellifera*, vision, grating resolution.

Introduction

The responses of bees to black and white gratings have been used previously for the measurement of resolution of the eye. In a careful study, which has been neglected in the recent literature, Hecht and Wolf (1929) plotted the relationship between the period of a regular grating and the minimum illumination that causes a response when the grating is moved. The bees turned in the opposite direction to the motion of the stripes placed beneath a transparent plate on which they walked. At an intensity of 4000 cd m^{-2} the bees could resolve a regular grating of period near 2° , but they required a period of 30° at a hundredth of this intensity. These authors inferred that the remarkable resolution in bright light is that of a single ommatidium and is also matched to the angle of 1° in the vertical direction between ommatidia at the front of the retina. This was not a study of learning or discrimination after training, and the bees detected the direction of the motion.

At about the same time, Baumgärtner (1928) studied the relatively poor discrimination between two rectangular patches of different colours. A rectangle 2 cm wide \times 1 cm high could be detected from a maximum distance of 12 cm, whereas one 1 cm wide \times 2 cm high could be detected from a maximum distance of 40 cm. This difference was attributed to the difference in the interommatidial angle in the two directions. Baumgärtner's result is sometimes quoted as evidence that the resolution of pattern perception is limited by the interommatidial angle.

There was nothing further until Srinivasan and Lehrer (1988) trained bees to discriminate from a distance between a

vertical and a horizontal black and white grating presented on a vertical surface. They found a limiting period near 2.5° . The trained bees were tested with targets of grey separately against vertical or, in different experiments, against horizontal gratings, and there was no difference in the resolution in the two directions. Based on this single result, they proposed that the resolution is limited by the field size of the individual receptors and not by the interommatidial angle, which is approximately 1° in the vertical and 2° in the horizontal direction at the front of the eye.

Srinivasan and Lehrer (1988) also used vertical and horizontal gratings composed of coloured papers that present negligible (2%) green contrast, or alternatively negligible blue contrast, at the edges where the colours meet, and found similar responses over a range of smaller periods. The blue receptors alone (with patterns with no green contrast) had a limiting period near 3.5° (see Fig. 4A). This is an interesting result because later Giger and Srinivasan (1996) trained with shuffled positions of the bars and found that the orientation cue is detected by green receptors only, and therefore requires green contrast. The earlier result was not explained, and the nature of the cue for the blue receptors was unknown.

These results were not incorporated into the literature, even by the authors themselves. For example, Lehrer and Bischof (1995) studied the resolution of coloured spots and interpreted their results in terms of the interommatidial angle: '*the finest possible resolution, the limit of which is set by $2\Delta\phi$* ', etc. This is apparently based on the assumption that the direction of the

motion of the grating is detected. One interpretation of the equal vertical and horizontal resolution was that bees analyse striped patterns with the oblique rows of ommatidia (Land, 1997), but vertical and horizontal gratings would then be indistinguishable.

This is all that is available concerning the resolution of grating patterns and their relation to the receptor array of the bee. A few studies of the resolution of coloured patches of different sizes (Lehrer and Bischof, 1995; Giurfa et al., 1996), which is quite a different task, produced conflicting results, but it is now clear that the resolution of coloured patches depends strongly on the stabilisation of the target on the eye, which in turn depends on green contrast (Horridge, 1999).

The limits of resolution of grating patterns can be explained if the cue is a difference in non-directional modulation detected by blue or green receptors, which both respond to black and white gratings. This hypothesis, that there is a difference in flicker, explains the discrimination between vertical and horizontal gratings with no green contrast and also why resolution is the same in the two directions. Near the resolution limit the orientations of the edges are not necessarily discriminated at all, but only the difference in the modulation as the eye scans first one target and then the other.

The purpose of the following work is to test this hypothesis, but the apparatus differs from that used previously, so it is necessary to repeat the earlier measurements and then make entirely new measurements with improved controls all on the same apparatus. In order to discover the actual cues used by the bees in each training situation, it is essential to test trained bees with a variety of patterns, including some that they cannot discriminate, giving an opportunity to exclude each possible cue.

Materials and methods

The apparatus

The methods used have been published many times (Srinivasan and Lehrer, 1988; Horridge, 1999). The present experiments were done under a roof with open front 3 m wide \times 3 m high in indirect sunlight. The top of the Y-choice apparatus is of clear PerspexTM and the walls are of white card (Fig. 1). The baffles, of transparent 'Artistcare Drawfilm', 0.13 mm thick, set in a cardboard frame 1 cm wide, control the angle subtended by the pattern at the bees' decision point. In the earlier studies, done without baffles, the bees could fly direct to the targets without being obliged to pause and look from a known distance.

The targets carry the patterns on white cards, which can be rotated. During training the target that displays the rewarded pattern and its reward are interchanged with the non-rewarded target every 5 min to prevent the bees from learning which arm of the apparatus to choose. In the figures the rewarded pattern is always shown on the left (labelled + at the top).

With the baffle at a distance of 27 cm, the square targets subtend an angle of approximately 55° at the point of choice. The bees required 20 or so visits to build up a discrimination.

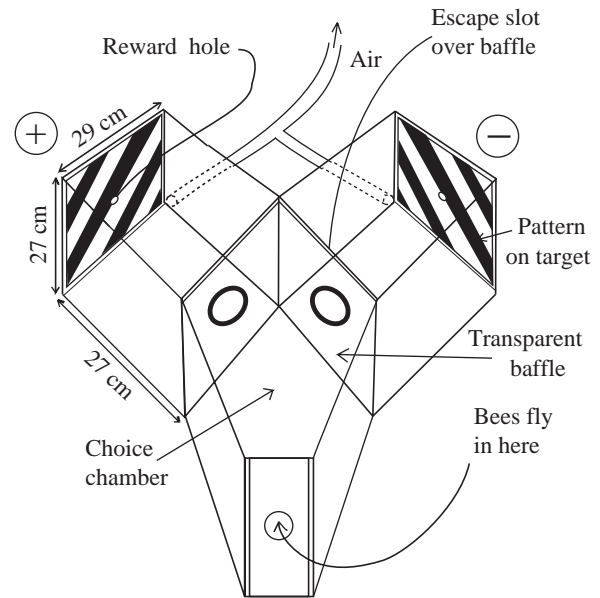


Fig. 1. The modified Y-choice apparatus. The bees enter at the front through a hole 5 cm diameter into a choice chamber, from which they can see both targets. They decide to enter through one of the baffle orifices 5 cm wide. To prevent the bees from learning which side to go, the targets and the reward change sides every 5 min. Odours are extracted by the air pipes. +, rewarded training pattern; -, training pattern without reward.

After an initial training period of 2–3 h, each first choice in each 5 min period was recorded while training continued. These results are labelled 'train'. In other experiments, labelled 'test', a different pair of patterns was substituted for those in the training, and the bees' first choices towards these were recorded in each period of 5 min. In the tests it was essential to give a reward, otherwise the bees continued to search in the Y-maze, and would not go away. All tests were done with one target rewarded and then repeated with the other target rewarded. Tests with gratings of different periods were interleaved during continued periods of training, so the trained bees did not become familiar with any of the tests. The same test pattern did not return for at least an hour. In some of the tests the bees failed to discriminate, so they learned nothing from the tests.

Calibrations

The grey and black patterns were made by a Hewlett Packard Laserjet 4M printer. The coloured papers, Nos 384 fawn and 595 light blue, were supplied by Canson Australia Pty, 17 Metropolitan Ave, Nunawading, Victoria, Australia. The reflectance spectra of the papers were measured as photon flux with a PC 1000 Fiber Optic Spectrometer (Ocean Optics Inc., Dunedin, FL, USA), near noon and again in the mid-afternoon. The detector, which has a spot field, was placed at the choice point of the bees and the papers at their usual place in the training and tests. The measurements covered a range from 290 to 830 nm with a mean resolution

of 0.52 nm. In the conditions of the experiments, in indirect light, there was negligible reflection of ultraviolet from these papers.

The calibration equipment generated digitized values, which were multiplied at 10 nm intervals, over the range from 380–620 nm, with the known spectral sensitivity curves of the bee receptor types, exactly as done by Giger and Srinivasan (1996). The products were summed to give the relative receptor excitation of the blue and green receptors, for each paper. The Canson 384 fawn/595 light blue combination gave negligible contrast to the green receptors. The emission curves of the papers, the relative excitation they generate at the receptors, and the contrasts have been recently published (Horridge, 1999).

Before each training or test that involved a plain grey target, a range of grey papers were compared with the target pattern to obtain one of the same luminance. This was done first by human eye with the targets placed side by side far away so that the gratings were not resolved.

Scoring and statistics

Each bee was identified by colours painted on the thorax and on the abdomen, and the criterion for the score was when the bee passed through the hole in a baffle. Unmarked recruits were removed. A record was kept of the first choice made by each bee in each period of 5 min, not the first choice of each arrival. Two statistical calculations were made. In the first, the fraction of correct choices was counted in each block of 20 choices. The standard deviation (S.D.) between 10–20 of these blocks was calculated as a percentage and placed after each score.

In the second method (van Hateren et al., 1990), S.D. was estimated from the value of $\sqrt{[p(1-p)/n]}$, where p is the fraction of correct choices and n is the total number of choices. This method assumes that there are no trends, that the individual choices are independent and they have a binomial distribution about the mean. The S.D. estimated from this formula is given in parentheses after each score. By this method a score of 57% correct based on 200 choices is twice the estimated S.D. away from the null (random) hypothesis of 50%.

Results

Training with no green contrast, vertical versus horizontal

The first task was to discover the cue used by the bees when horizontal and vertical gratings are discriminated although green contrast is negligible. A group of bees was trained to discriminate between horizontal (rewarded) and vertical (unrewarded) gratings of period 14° made with blue (Sanson 595) and fawn (Sanson 384) papers giving less than 2% contrast to the green receptors where the papers meet (Fig. 2A). The patterns were asymmetrical about the central reward hole and were turned through 180° every 5 min. Learning was rapid despite the alternation of bar positions and lack of green contrast. The result after 2 h training was $74.5 \pm 3.6\%$ (3.1%) correct for the next 200 choices, and improved to over 75%

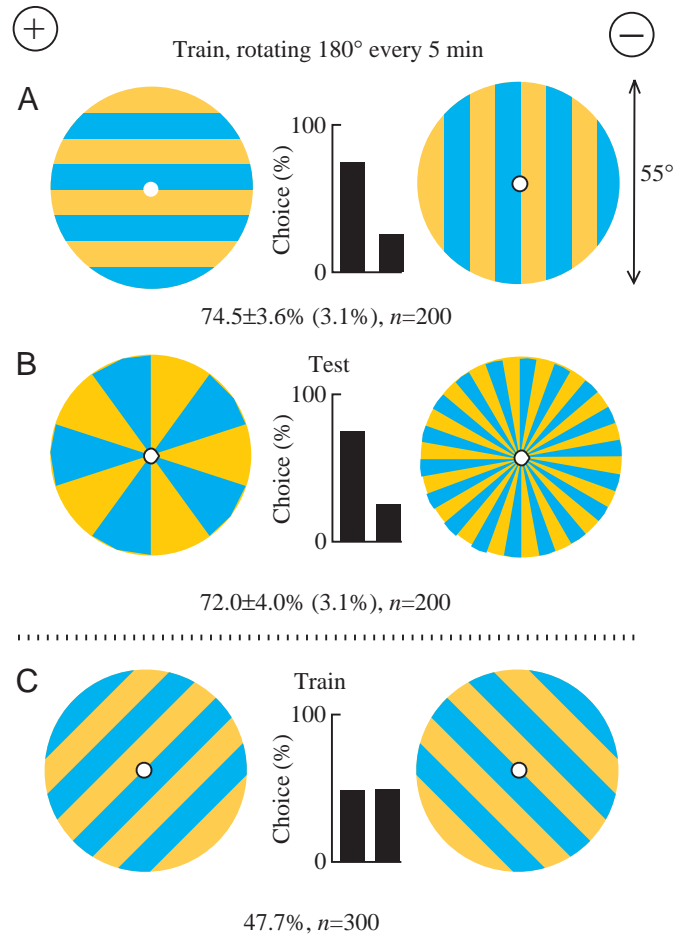


Fig. 2. Training without green contrast. (A) Discrimination between horizontal and vertical coarse gratings, both rotated by 180° every 5 min to shuffle bar positions. (B) Test on coarse *versus* fine sector patterns. (C) Train and test on the same patterns as in A, rotated by 45° . +, rewarded training pattern; –, training pattern without reward.

correct between subsequent tests. Green contrast is not necessary for the discrimination.

To test whether the cue is related to size or spatial frequency, the trained bees were tested with a pattern of 10 sectors *versus* a pattern of 36 sectors, both made in alternate sectors with the blue and fawn papers that gave no green contrast (Fig. 2B). The result was 72.0% in favour of the larger sectors. Clearly, in a forced choice, the bees discriminated something related to size or modulation.

The trained bees were also tested with a horizontal *versus* a vertical black and white grating of period 4° with a result of 60.5% correct choice, $n=200$ (not illustrated), in favour of the vertical (previously unrewarded) grating. They had obviously not learned the edge orientations, and in the forced choice they probably reverted to their spontaneous preference. When tested with a black and white checkerboard of period 8° *versus* a grey target (45% black), the result was 52% in favour of the check (not illustrated), showing that the bees had not relied upon a simple difference in modulation. The horizontal bars in the training pattern were larger in the horizontal direction than the

vertical bars, and the results suggest that when trained on a period of 14° without green contrast, something coloured and related to size is detected with the help of horizontal scanning movements in flight. This is an unexpected interpretation that could apply to earlier work. This cue was avoided in the next experiment.

Training with no green contrast, oblique gratings

In this experiment, the patterns presented no difference in modulation as the bees scan in the horizontal direction. A new group of bees was trained to discriminate between two orthogonal gratings of period 14° with no contrast to the green receptors. The rewarded grating was oriented at 45° , the other at 135° to the vertical (Fig. 2C). The patterns were asymmetrical about the central reward hole and were turned through 180° every 5 min to prevent the bees learning the bar location. Although training continued all day, the final result was 47.7% correct choice, $n=300$.

In this case, there was no orientation cue, no modulation difference, no difference in size of areas that can be detected by scanning motion, no average colour difference, and no fixed positions of areas of different colour. Of course, the bees saw the targets and the colour but they did not discriminate the patterns.

Horizontal versus vertical, black and white gratings

To confirm the earlier finding (Srinivasan and Lehrer, 1988) with a different procedure, a new group of bees was trained to discriminate between a horizontal (rewarded) and a vertical (unrewarded) black and white grating of period 5° , with the patterns rotated by 180° every 5 min to prevent the bees learning the bar positions (Fig. 3A). In the earlier work the patterns were fixed and there were no baffles to make the bees pause in flight. After 2 h training the result was $70.5 \pm 3.4\%$ (3.2%) correct over the next 200 choices. The trained bees were tested with black and white horizontal gratings of various periods *versus* plain matched grey targets (45% black). There was no discrimination with a period of 2.0° , poor at 2.5° , but with larger periods the performance improved rapidly (Fig. 4B).

Similarly, new bees were trained with the vertical (rewarded) *versus* the horizontal grating of period 5° and tested with black and white vertical gratings of various periods *versus* plain grey targets (45% black). The results with horizontal gratings were similar to those with vertical gratings, both tested against grey targets (Fig. 4B). When trained on a period of 5° the resolution was independent of direction, as previously found with different apparatus. Whether the cue in this case was edge orientation or modulation, or something else, remains to be determined.

Oblique black and white gratings

The next experiment measured the resolution when there is green contrast but no difference in modulation caused by scanning. A group of bees was trained to discriminate between

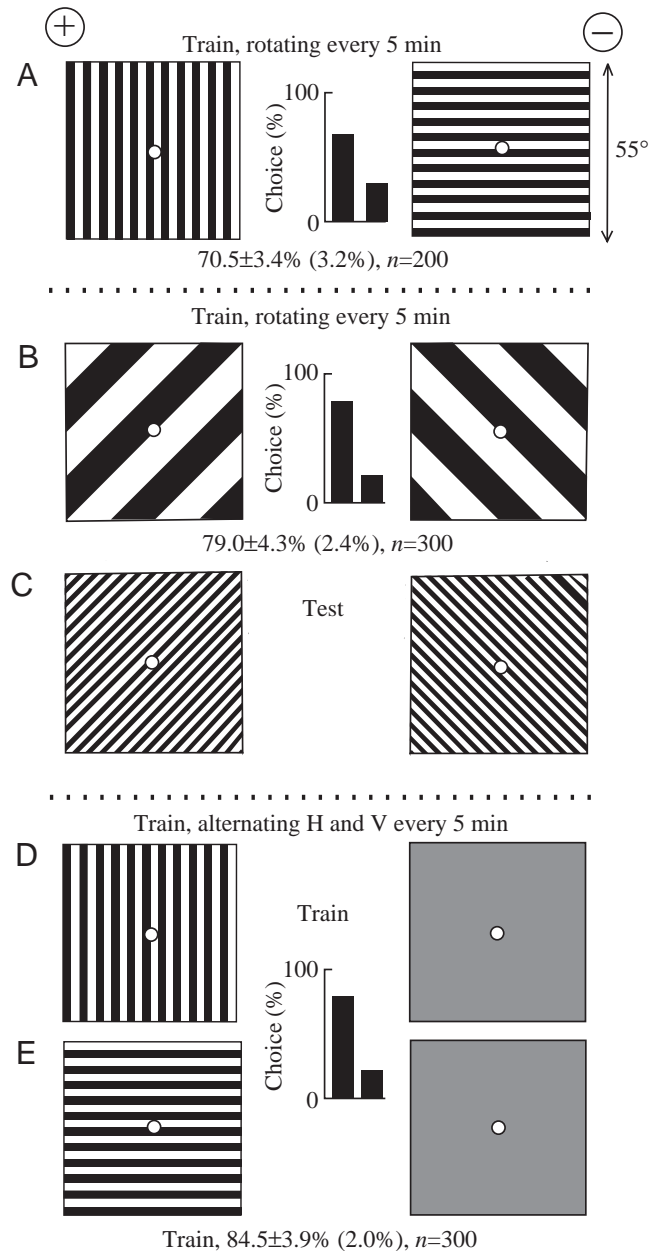


Fig. 3. The gratings used in the resolution tests. (A) The bees were trained on vertical *versus* horizontal gratings of period 5° with baffles in place. They were tested with vertical or horizontal gratings of various periods. The results are given in Fig. 4B. (B) New bees were trained on coarse oblique gratings or (C) with fine gratings of various periods. The results are given in Fig. 4B. (D,E) The bees were trained on a grating of period 5° *versus* grey. The grating alternated between horizontal (H) and vertical (V) every 5 min to remove the orientation cue. The results of tests with H gratings *versus* grey and V gratings *versus* grey are given in Fig. 4C. +, rewarded training pattern; -, training pattern without reward.

two black and white gratings of period 18° , one at $+45^\circ$ to the vertical, the other at -45° (Fig. 3B). The targets were rotated by 180° every 5 or 10 min so that the bees did not use the locations of the bars as cues. After 3 h training the result was

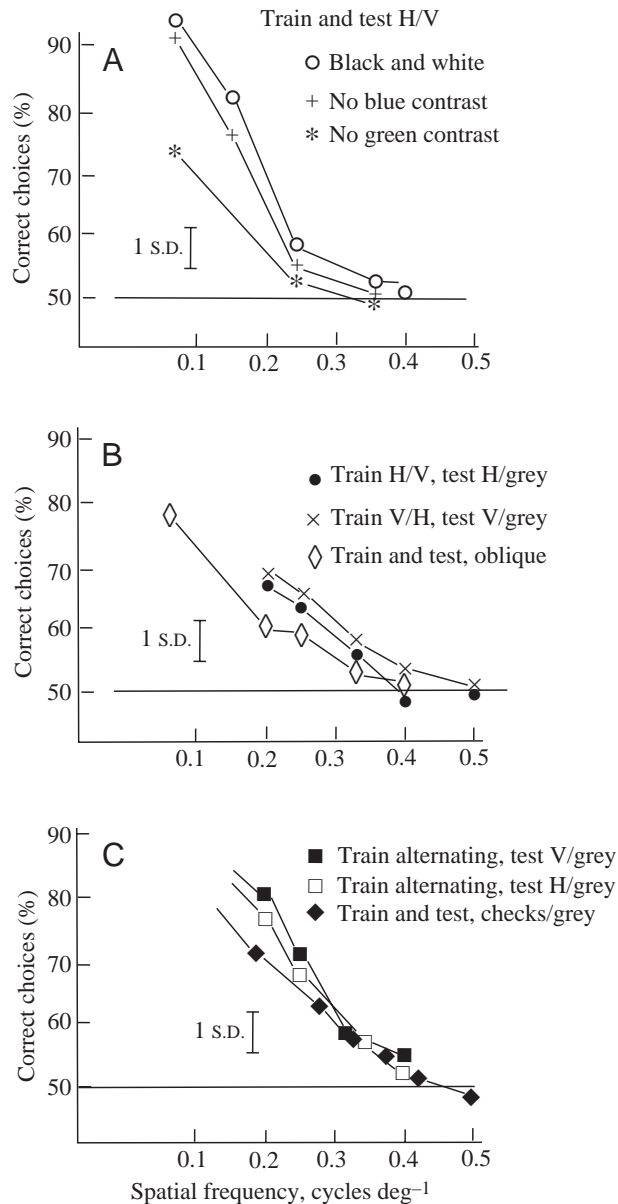


Fig. 4. Summary of results. (A) Data from Srinivasan and Lehrer (1988). (B) Data from Fig. 3A–C. (C) Data from Fig. 3D,E. Values are means \pm S.D. calculated for $n=200$. H, horizontal; V, vertical.

79.0 \pm 4.3% (2.4%) correct choice, $n=300$. The trained bees were tested with equally spaced black and white gratings of various smaller periods in interleaved tests. The performance dropped to 57.5% correct at a period of 4°, 53.0% at 3°, and 51.5% at 2.5° (Fig. 4B). Significantly, the resolution with oblique gratings was not as good as that with horizontal or vertical black and white gratings *versus* grey.

It is difficult to imagine a difference in the modulation caused by gratings at 45° and those at 135°, so it is probable that the cue is edge orientation, not receptor modulation. This point was tested by presenting the bees trained on black and white oblique gratings (Fig. 3B) with a test checkerboard of period 4° *versus* a plain grey target (45% black). The result

was 51.5% correct, $n=200$, showing that the bees did not rely on a modulation cue. For the green receptors and oblique black and white gratings the orientation cue in isolation appeared to be visible down to grating periods of 3.5°, which is not as good as the discrimination of modulation.

Alternating horizontal and vertical gratings

The discrimination between horizontal and vertical black and white gratings (Fig. 3A) could be based on the difference in orientations of edges or differences in the receptor modulation caused by the two patterns, but we found indications (Fig. 4B) that the resolution is poor when the modulation cue is removed. To test the resolution of black and white gratings when orientation is eliminated, we required a new regime that trains the bees to ignore the edge orientations.

A new group of bees was trained with a plain grey target (45% black) *versus* a black and white grating of period 5° that was alternated between vertical and horizontal every 5 min (Fig. 3D,E). A small period was selected because it was easy to match with a grey level of equal luminance. The task was an easy one; after 3 h training the result was 84.5 \pm 3.9% (2.0%) correct choice, $n=300$. In two separate series of tests, the trained bees were tested with black and white gratings with a range of periods *versus* the grey target, with the gratings vertical (as in Fig. 3D) or horizontal (as in Fig. 3E). As the period was made smaller, the gratings were less easily discriminated from the grey in the tests (Fig. 4C). The resolution was similar to that after training with two black and white horizontal and vertical gratings (Fig. 4A,B), and similar in vertical and horizontal directions tested separately, but the cue cannot be orientation.

Taken together with the previous experiment, this result shows that orientation and modulation are separate cues, either of which is effective, depending on the training, and that the discrimination of gratings does not rely only upon an orientation cue in oblique rows of facets.

Train and test on checkerboards *versus* grey

Another training routine that avoided orientation as a cue made use of checkerboards. A group of bees was trained to discriminate between a checkerboard of period 5.6° (between diagonal lines of squares) *versus* a plain grey target of equal luminance (47% black). This was an easy task and the performance after only 3 h training was 74.0 \pm 4.2% (3.1%) correct choice, $n=200$. The trained bees were tested with checkerboards of smaller periods *versus* a plain grey target (47% black), and the results plotted in Fig. 4C. Discrimination was lost when the period between the diagonal lines of squares approached 2.5°. The cue cannot be orientation, and in this case the bees were not trained to ignore edge orientation.

Discussion

Receptor fields and spacing

At the front of the honeybee eye the interommatidial angle of approx. 2° in the horizontal direction is twice that of approx.

1° in the vertical direction (Seidl and Kaiser, 1981). For the worker honeybee, the measured width of the receptor field at the 50% sensitivity contour is near 2.5° (Laughlin and Horridge, 1972) or 2.6° (Labhart, 1980) and is symmetrical. These electrophysiological measurements could be an overestimate because the optics may have been damaged by the recording and the eyes were not necessarily fully light adapted. In any event, the eye is not uniform and these are selected values. However, the behavioural measurements agree with a minimum field width near 2.5° in the vertical and horizontal directions, even when the orientation cue is eliminated from the training. Srinivasan and Lehrer (1988) inferred that the resolution for vertical *versus* horizontal gratings is limited by the minimum detectable modulation, not by the angles between adjacent ommatidia, but they made no effort to test this hypothesis. The new experiments show that with oblique gratings with green contrast (Fig. 3B), the bees can also use orientation as a cue down to periods near 3.5° (Fig. 4B). With horizontal *versus* vertical gratings, and no green contrast (Fig. 2A), they can use the modulation with blue contrast, or the horizontal size of broad bars as a cue if all else fails (Fig. 4A). With oblique gratings with no green contrast (Fig. 2C), they detect no difference, which casts doubt on whether they detect anything about pattern except the known cues.

The cues, and therefore the resolution, depend on the task

The resolution depends on what the bees use for the visual cue. At least four kinds of cue are already known, with corresponding values of the resolution. The common statement that the resolution of a compound eye depends on the interommatidial angle has two sources. The first is the principle that the facets of the compound eye divide the outside world into an array of little windows, and, when the rhabdomeres are fused, each detects the photon flux in its field of view. The spacing between sampling stations must be half the period of a regular grating that is reconstructed by the visual system. There is no evidence, however, for reconstruction.

Secondly, if the resolution test depends on the detection of the direction of motion, as in the optomotor response, the motion is detected from the modulation at a receptor followed by that at an adjacent visual axis (Götz, 1965). The angle between receptor axes is therefore an essential part of the spatio-temporal filter that detects the direction of motion.

If the cue at the resolution limit is the difference caused by the two targets in the modulation of the individual receptors, there is no need for the edge orientation or the direction of motion to be discriminated, or the pattern to be reconstructed. The discrimination between two gratings then is ultimately limited by the acceptance angle of the receptors, not the angle between their axes. This explains why bees trained without an orientation cue have the same limit near 2.5° when horizontal and vertical gratings are tested separately against grey, although the corresponding interommatidial angles have a ratio of 2:1.

Gratings at 45° *versus* 135° generate the same modulation

from the bees motion in flight, so the only cue then available is the edge orientation, which requires the detection of edge direction from at least two simultaneously modulated ommatidia, and green contrast. The resolution limit for the orientation cue in a grating can only be demonstrated when a modulation difference has been eliminated as a cue, and the grating period is then near 3.5°.

Finally, when the cue is the discrimination between two positions (Baumgärtner, 1928) or the detection of a patch of colour *versus* a blank target (Lehrer and Bischof, 1995; Giurfa et al., 1996), the limit of resolution depends on a more complicated processing that requires several ommatidia. This is quite a different process that is strongly dependent on green contrast to help stabilize the eye on the target (Horridge, 1999).

One lesson from these results is that bees use an extreme abstraction of the pattern, single receptor modulation, as the cue when learning to discriminate between two patterns. They do not remember, and probably do not see, a grating as a pattern of repeated bars.

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