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Pattern discrimination by the honeybee: disruption as a cue

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Abstract The discrimination of pattern disruption in freely flying honeybees (*Apis mellifera*) was examined. Bees were trained to discriminate at a fixed distance between a regularly repeated black/white pattern and the same pattern at a different magnification in targets of the same angular size. The locations of areas of black were regularly shuffled to make them useless as cues. The results of the experiments indicate that the bees discriminate the disruption of the pattern as a whole, irrespective of the actual pattern. Bees trained to prefer a larger period transfer to an even larger period, when given a forced choice with a pair of patterns of differing disruption from those they were trained on, as if their spontaneous preference has not been overcome. Bees trained to prefer a smaller period, however, prefer the former negative pattern rather than transfer to an even smaller period. These results show that the bees do not rely solely on learning the absolute period of a pattern nor the relative disruption of two patterns, and they are confused when these two cues conflict in tests with unfamiliar targets. Bees can discriminate between fields of view that differ in average disruption as a generalized cue, irrespective of pattern.

Key words Vision · Spatial frequency · Honeybees · *Apis mellifera* · Pattern discrimination

Introduction

In experiments with fixed patterns presented on a vertical surface, von Frisch (1914) found that bees discriminate between a flower shape with four and one with

many petals, but that they are unable to discriminate between a variety of geometrical shapes such as a square, a round disc and a diamond, or between a check of triangles and one of squares. The patterns were stationary over the whole learning period and the criterion for the choice was the landing of the bees on a target. Even at that time it was clear that bees do not see pattern as we do, but nothing was said about the possible cues that could be used by the bees. For von Frisch's student, Baumgärtner (1928), pattern disruption was not a proposed cue either. In fact, Baumgärtner reported that a flower pattern with four petals could not be discriminated from one with five petals if the petal location is randomized by rotation of both targets.

For the past 65 years, however, it has been proposed that trained honeybees can make a discrimination between two targets because the cue can be a difference in modulation induced at the receptors as the compound eye moves relative to the two images. The experimental basis for disruption as a cue was the extensive work of Hertz (1929a,b; 1931) who presented many patterns of similar size on a flat white table and switched them about at intervals so that bees could not learn their locations relative to local landmarks. With this arrangement, Hertz found that bees discriminate consistently when the patterns differed in length of edge. The shuffling around of the areas of black left only the size, disruption of the patterns and radially symmetrical cues available to the bees, but this point was not made until recently (Horridge 1996b). The idea of disruption as the cue was further established by Zerrahn (1933) who used a variety of patterns of similar size, also presented on a flat table. Zerrahn proposed that the critical cue for spontaneous preferences of naive bees, as well as for discriminations by trained bees, was the ratio of area of black to length of edge. Of course, this formula, and others like it, is not a visual processing mechanism, but is a way to summarize the patterns that the bees distinguish. It is hard to believe that bees actually make these measurements and at the same time fail to discriminate many geometrical shapes. The idea of seeing

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by pattern disruption was presented by Wolf (1933) who later found that bees were attracted to flowers in motion (Wolf and Zerrahn-Wolf 1937), which may or may not have increased the flicker at the eyes of the bees in flight. Later, the high flicker fusion frequency of bee's eyes was explained as an adaptation to detecting (but not necessarily resolving) a rapid succession of edges during flight (Autrum 1957).

Considering the controls required to demonstrate spatial frequency as a cue, it is clear that: (1) the point of choice must be at a known range from the target otherwise spatial frequency is meaningless, (2) the areas of black must be shuffled to prevent their locations being used as cues, (3) the bees must not see other bees in the tests, and (4) innate preferences and patterns with unknown cues must be avoided. von Frisch (1966) accepted differences in flicker as cues in discrimination with hardly a mention of his own earlier results, or the lack of controls. Except for Anderson (1972, 1977), who found that a regularly repeated fixed pattern could be discriminated from the same pattern at a different scale, there was no critical experiment that demonstrated unambiguously that the disruption was the cue because there were always other differences between the patterns. However, in Anderson's experiments the criterion for a choice was the landing of the bee on the target so that the bees' decisions were taken at a very short range, at which only local features could be used. One of the purposes of this study is to test the disruption theory when the above precautions are observed.

Even in the earliest work, not all patterns with similar length of edge or area of black were confused by the bees even when presented horizontally and shuffled in location (Hertz 1933), so there were other cues. In quite different experiments on the discrimination of average orientation in large patterns subtending 130° at the point of choice, Wehner and Lindauer (1966) carefully avoided different contour lengths or pattern disruption by using two identical patterns, one rotated relative to the other, but the patterns were very large and fixed in one position for the whole learning session. As shown by later work (Giger and Srinivasan 1995; Horridge 1996b), the bees could have used the retinal locations of areas of black as cues, even when the patterns were of different numbers of sectors (review: Wehner 1981; and Fig. 67 therein).

For vision in the natural world or for discrimination between two natural objects, pattern disruption is not an efficient cue because the angular spatial frequency at the eye is proportional to range, the ratio of edge to area is a very insensitive measure of shape, a reliance on disruption throws away the spatial lay-out of the image, and natural objects differ greatly from each other in ways other than spatial frequency. Only if the bee fixates on a flower at a constant range could it use the global disruption of the flower pattern as a cue, and there are so many other possible cues. No doubt for these reasons, in an extensive review of the subject Wehner (1981) was less than enthusiastic about the flicker theory of insect

vision. The flicker theory persisted in standard texts (Wigglesworth 1965; von Frisch 1966), but for 60 years the main difficulty has been the lack of new data. Suggestions about a function for the discrimination of disruption need to be supported by controlled experiments. As quite a different issue, a high temporal modulation frequency has long been recognized as a signal for landing, with or without learning, as suggested by Zerrahn (1933) and Anderson (1977) among others.

In recent work, the use of pattern disruption as a cue has been avoided by the randomization of locations and sizes of targets (Lehrer et al. 1988; Horridge et al. 1992), by use of four equal bars in all patterns (Horridge 1996a), by the use of identical patterns, one rotated relative to the other (Horridge 1996b), by randomizing numbers and positions of bars in gratings (Srinivasan 1994), or of sectors in radial patterns (Horridge and Zhang 1995). In previous work on disruption as a cue (Hertz 1933; Zerrahn 1933; Anderson 1977), other possible cues were not randomized.

In the experiments to be described, the bees were trained to discriminate between a pattern and the same pattern at a different scale, but avoiding constant locations of areas of black, and making use of the fact that the average brightness is unchanged by change of scale in regularly repeated patterns.

Materials and methods

The training and tests were carried out in the standard Y-choice apparatus of Srinivasan and Lehrer (1988) which offers the bees a choice between two targets at a fixed distance of 27 cm from the choice point. The walls of this apparatus are of white card, the top is of clear Perspex. At the entrance to each arm the original design was modified by the addition of a baffle with a hole 5 cm in diameter at its centre surrounded by a black annulus 0.5 cm wide (Fig. 1). The baffles, of transparent 'Artistcare Drawfilm' 0.13 mm thick, set in a cardboard frame 1 cm wide, force the bees to pause and then pass through the central hole, which becomes the decision point, and also allow the experimenter to make sharp decisions about the bees' choices. As shown by a reduction in the standard deviations since they were adopted, the baffles improve the performance, as if they make the bees stop and look at the targets. After feeding, the bees can exit the maze by flying through the hole or by walking under or over the baffle. The apparatus was placed in an open shed outside with the targets facing bright daylight.

Honeybees from a nearby hive select one of the two targets while in flight in the central chamber. Each bee that enters is identified by its colour marking, and the criterion for a score is when the bee first passes a baffle. With the baffles at a distance of 27 cm, the round targets of 25 cm diameter subtend an angle of 50° at the bee's eye when the bee is about to pass through. The targets have a hole 2 cm in diameter at the centre, for access to the reward on the side defined as positive and leading to a blind tube on the negative side. The reward is a fresh solution of sucrose of a strength that keeps the bees making regular visits without recruiting many others. The side of the positive target and the reward with it are interchanged every 10 min (or sometimes after 5 min) to prevent the bees from learning which side to visit, but the rewarded pattern (+) is always shown on the left in the illustrations.

All patterns were drawn by computer and printed in black on white paper of a constant quality. The positive and negative patterns were of the same type, differing only in scale. In different experiments (except those illustrated in Figs. 2 and 11) four types

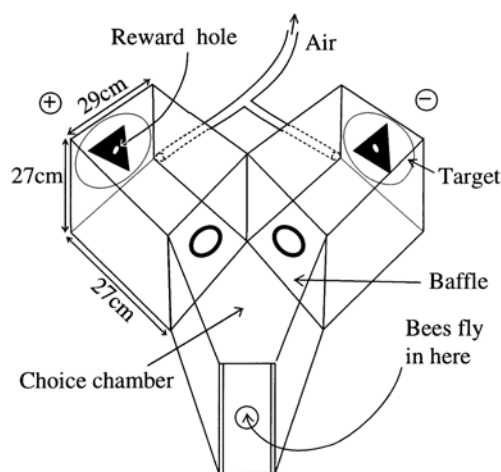


Fig. 1 The modified Y-choice apparatus, which stands outside under a roof. The bees enter through a hole 5 cm diameter in the front to a choice chamber from which they can see both targets. They normally spend some time looking through the transparent baffles until they decide to pass through one of 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

of patterns were used: linear gratings, sector discs, chequerboards, and spirals. In all the experiments there was a change of both positive and negative pattern every 5 min during the training and again during the tests, so that areas of black are not presented in constant locations. The grating and square patterns were prepared with different phase relative to the central hole, while the sectors and spirals were rotated randomly at every change of target, as illustrated in the figures. This strategy means that the positive and negative patterns differ only in scale or degree of disruption, and the bees are taught to pay no attention to the locations of areas of black in the pattern, or to the existence of a particular detail in one pattern and not in the other.

After an initial training, usually for 2 h, the performance was measured while training continued. These results are labelled "Train and test" in the illustrations. The trained bees were then repeatedly tested, for intervals of only 5 min on each side between continued training, with other pairs of patterns, first with the positive pattern on one side and then with the sides reversed. These tests were introduced at random between longer intervals of continued training. In most cases, the tests were repeated with the positive and negative patterns reversed, to eliminate any possible effect of rewarding only one of them during the tests. These results are labelled "Test". The bees have time to make only one rewarded visit in any one test interval, whereas they require 20 or so visits to build up a memory of a single pattern. The test intervals are very short compared to the time required to train bees with two patterns, and previous studies, keeping separate the results before and after the tests as at the foot of Fig. 4, show that the tests have a negligible effect on subsequent performance. The reason is not hard to find. When the positive and negative patterns are reversed, in any learning experiment, it takes about 2 h of continuous training to get back to a choice of 50%, i.e. no preference (Horridge and Zhang 1995). It is quite a different matter in the learning of landmarks, sometimes in a single trial (Horridge 1996d). As another control, when the reward was omitted altogether in the tests, there was no effect on the test results (Horridge et al. 1992) but the bees continue to search in the apparatus and disturb newly arriving bees.

Previous work has shown that bees can resolve the gratings and bars used here (Srinivasan and Lehrer 1988). The resolution of the bee's eye for regular black and white stripes of a parallel grating is adequate to give at least 65% correct choice at 4° per stripe period, which falls to 50% at 3° per repeat period, measured with the same Y-choice apparatus with no baffles. A grating of period 4° is drawn

to scale in Figs. 2, 3, 9 and 10 for comparison with the pattern period at the scale at which those figures are reproduced.

The bees are individually marked and a separate record is kept of the score of each. The results from each pair of patterns are kept separate. With each pair of patterns, 6–20 tests were conducted, each of two periods of 5 min. For each test, the percentage of correct choices was calculated from the total number of choices, and from the percentages obtained in all tests, the mean value and the standard deviation was calculated for each pair of patterns. The group of six to ten bees make a total of 10–20 visits in each interval of 10 min between the changes of side during the training, coming more frequently on hot days. There are no discernible differences between the performances of individual bees but some bees come more often than others. The significance of the differences between the test results and the theoretical expectation of 50% was calculated by the χ^2 test with one degree of freedom. This is a severe test that gives minimum values of the significance. In the illustrations, the percentage of correct choices and its standard deviation, as well as the statistical significance of the result (if in doubt) is given for each pair of patterns.

Results

The necessity of randomizing the distribution of black areas

The first set of experiments illustrates the fact that bees can use the location of black areas with targets subtending 50° at the point of choice, and justifies the measures taken in the other experiments to randomize the spatial distribution of black and white areas in the patterns.

A group of bees was trained to discriminate between a large black triangle and the same triangle oriented upside down in the Y-choice apparatus, which forces the bees to look at the target from a fixed point before they pass the baffle. The reward hole is at the centre of the triangle. After training for 2 h, the bees still cannot distinguish between the patterns, although both targets are fixed and the side of the triangle subtends 35° from the point of choice (Fig. 2a). However, when the triangles are moved by 3 cm (Fig. 2b), the bees soon learn to discriminate. After 2 h of training the result was $75.3 \pm 2.6\%$. The trained bees, tested at the same time on the original task, failed to discriminate (Fig. 2c). This result confirms that the average location of the black area is a powerful cue and that bees cannot discriminate between some simple geometrical shapes, although, of course, there are many other pairs of shapes that they can discriminate for other reasons.

A new group of bees was trained to discriminate between a fixed horizontal grating and the same grating moved by one half period, with a period of 25° and the target subtending 50° at the point of choice (Fig. 2d). The result was $66.9 \pm 2.9\%$. The bees were then tested with a single black disc of diameter 4 cm, placed above or below the reward hole (Fig. 2e). They perform almost as well as with the original pattern, showing that they use the location of black relative to the reward hole as a cue. The result was $61.8 \pm 3.2\%$. The scores are not as high as in similar experiments with larger targets (Horridge 1996b).

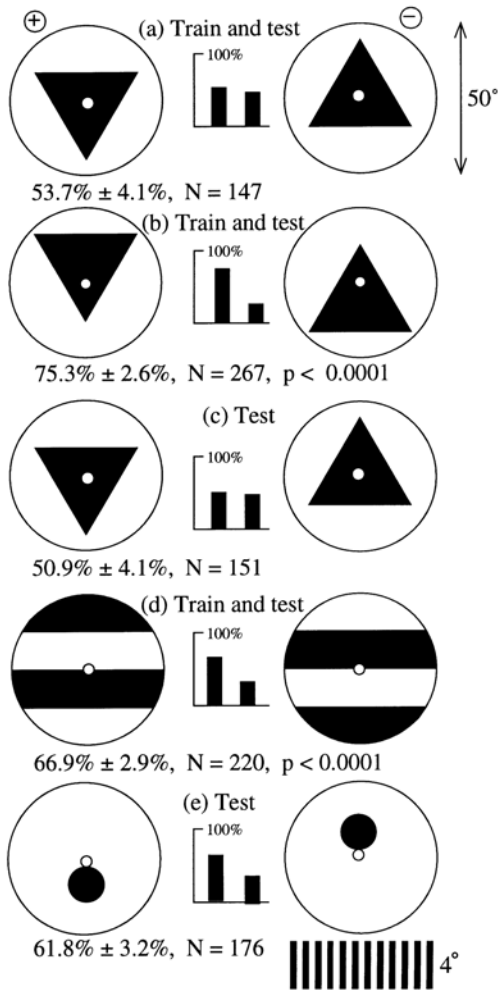


Fig. 2a-e The reason for the control of the locations of the areas of black in the patterns. **a** Bees cannot discriminate a fixed triangle from itself inverted. **b** If the triangles are displaced by 3 cm, the bees can discriminate the two patterns. **c** The bees trained in **b** fail to discriminate when tested with triangles on the same level, as in **a**. **d** Bees discriminate between a fixed grating of period 8.3° and the same pattern displaced by half a period. **e** Bees trained on **d** discriminate correctly when the cue is the location of a black disc

These experiments show that if two different patterns are presented in fixed positions, the bees use the location of a large area of black as a cue. The minimum required difference has not been measured, but this cue must be made useless in all the following experiments, or else the bees can use it.

Training on regular vertical gratings

A group of bees was trained on a positive vertical grating of period 6 cm versus a negative one of a larger period 12 cm, with random change of phase between four possibilities every 5 min (Fig. 3a). After training for 2 h the result was 72.1 ± 3.8% in favour of the smaller period. The trained bees were then tested 12 times with

period 6 cm positive and period 4 cm negative (Fig. 3b), and 12 times with period 4 cm positive versus a period of 6 cm negative (Fig. 3c). The combined result was 58.8 ± 4.0%, in favour of the period of 6 cm, revealing a weaker preference than before for the pattern they were trained on. This result suggests that, during training the bees have learned to prefer not only the smaller of the two periods, but also the absolute period of the rewarded pattern. When the rewarded pattern is tested against an even smaller period, the preference for whichever test pattern is smaller conflicts with the absolute value of the period learned.

In a similar experiment (not illustrated), a new group of bees was trained on a positive vertical grating of 6 cm period versus a negative one of larger period of 10 cm with random change of the phase of the patterns every 5 min. After a brief training of 1 h the result over the next 2 h was 67.6 ± 3.5% (*n* = 180, *P* < 0.01) in fa-

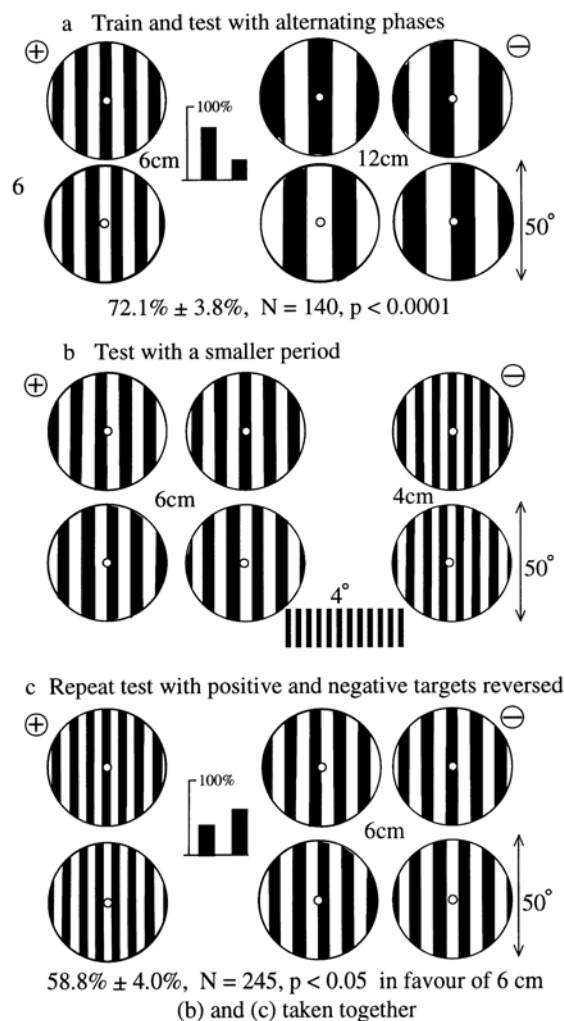


Fig. 3a-c Trained bees do not rely on the relative period as a cue. **a** The positive target has the smaller period. **b** The trained bees are tested with a still smaller period versus the previous positive target. **c** The test is repeated with the other target rewarded

avour of the smaller period. Between intervals of continued training, the trained bees were tested 12 times on 6 cm positive period with 4 cm negative, and 12 times with 4 cm positive versus 6 cm negative, again with random changes in phase. The combined result of these tests was $59.9 \pm 3.5\%$ ($n = 246$, $P < 0.01$) in favour of the 6 cm period, showing again some evidence that the bees learn the absolute period and the relative period of the two patterns. As a result, the preference for the 6-cm period is reduced when this previously rewarded pattern is tested against an even smaller period.

In the converse experiment, a new group of bees was trained on a positive period of 6 cm versus a smaller negative period of 4 cm with random change of phase between four possibilities every 5 min (Fig. 4a). The result was $74.2 \pm 3.5\%$. When tested with a period of 10 cm positive versus 6 cm negative, they prefer the former, although the 6-cm pattern was the one rewarded in the training. The result was $72.2 \pm 4.4\%$ (Fig. 4b). In

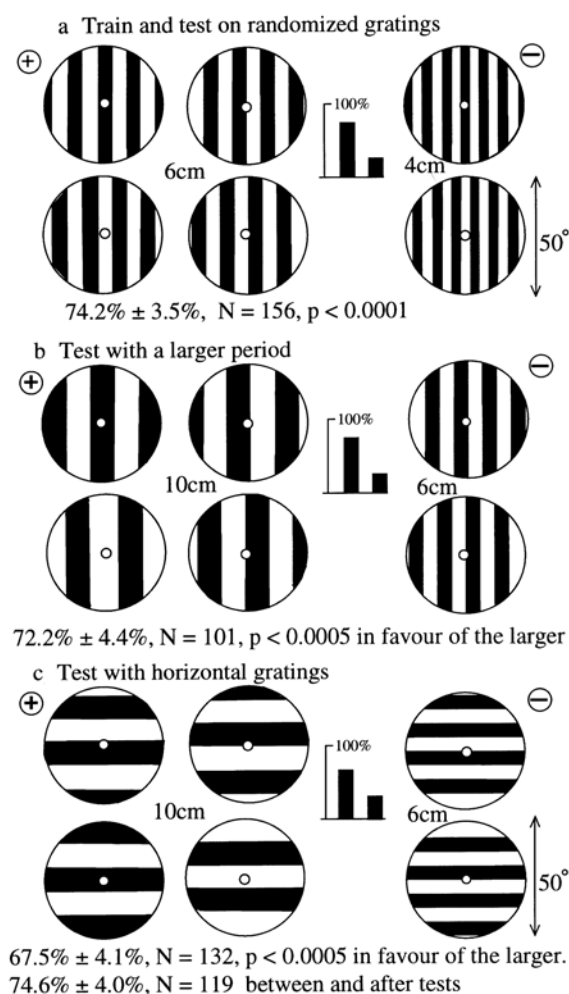


Fig. 4a–c Bees trained to the larger period transfer to a still larger period. **a** The positive target has the larger period. **b** The trained bees are tested with a still larger period versus the previous positive target. **c** The test is repeated with the gratings horizontal

this case the bees have not learned the absolute period of the rewarded pattern; in fact, it is questionable whether they have learned anything at all because the results could be due to the spontaneous preference of the bees when decisions are made at some distance (Lehrer et al. 1995). This group of bees trained on vertical stripes, was also tested with horizontal gratings with a period of 10 cm positive versus 6 cm negative, again with random change of phase every 5 min (Fig. 4c). The result was $67.5 \pm 4.1\%$ in favour of the larger period. Thus, the preference for the larger period may be due to an innate preference. The bees appear not to be disturbed by the change in pattern, and maybe any appropriately disrupted pattern would be accepted, as shown in Fig. 5. Between tests in Fig. 4, the continuation of the training program yielded a result of $74.6 \pm 4.0\%$ ($n = 119$) in favour of the 6-cm period.

To test further the tolerance of the bees to a change in pattern, a new group of bees was trained on randomized-phase vertical gratings of period 6 cm positive and 4 cm negative (Fig. 5a). The result was $88.3 \pm 2.1\%$ after 3 h of training. These trained bees were then tested with targets of six sectors versus eight sectors, with a result of $60.1 \pm 4.9\%$ in favour of six sectors (Fig. 5b). Note that they selected the lower spatial frequency, and that the standard deviation is larger when unfamiliar patterns are offered. The same trained bees also selected

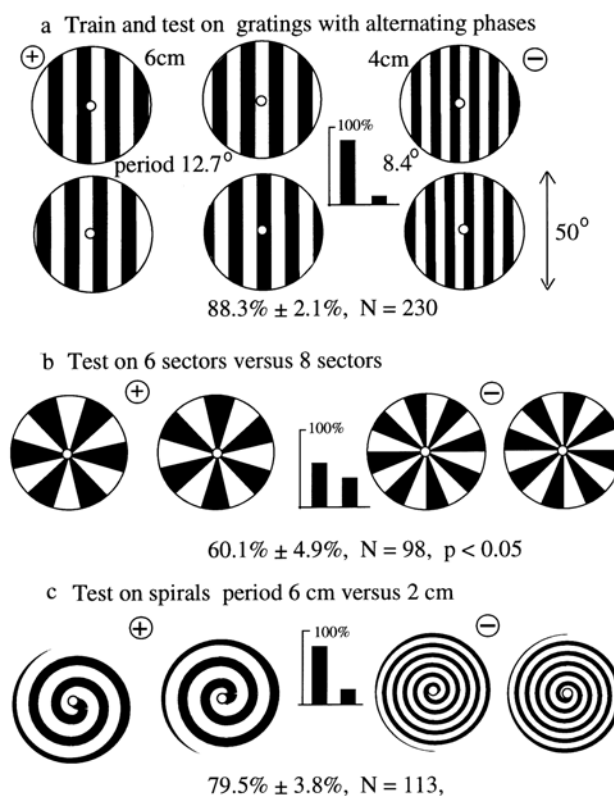


Fig. 5a–c Trained bees transfer their discrimination to other patterns. **a** The positive target has the larger period. The trained bees transfer correctly to the larger period in **(b)** sectors and **(c)** spirals

the coarser of two spirals, one of period 6 cm versus one of period 2 cm with the result $79.5 \pm 3.8\%$ (Fig. 4c). Again, bees easily transfer their preference, as if they are not concerned with the actual patterns.

Training on regular radial sectors with random phase

Regular sector patterns lend themselves to the randomization of phase, which is done by rotating the target every 5 min (Fig. 6a). A group of bees was trained on targets of 6 black sectors positive versus 12 black sectors negative, with random phase. The result after 2 h of training was $75.4 \pm 3.1\%$. The trained bees were then tested 12 times with 4 black sectors positive versus 8 sectors negative and 12 times with 8 black sectors positive versus 4 sectors negative (Fig. 6b). The result of all 24 tests combined was $63.1 \pm 3.6\%$ in favour of the target with 4 sectors, although the bees were trained to choose 6 sectors. As with linear gratings (Fig. 4), bees

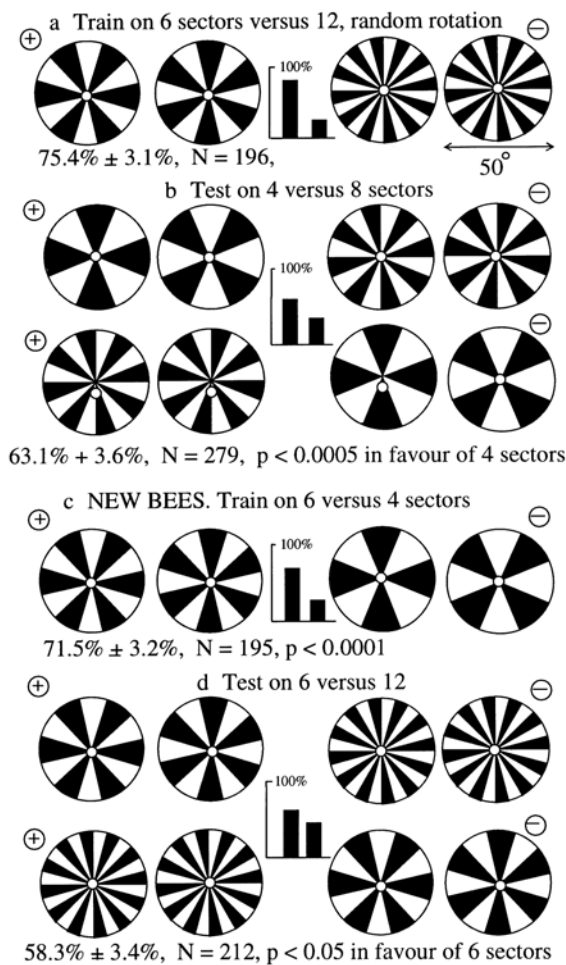


Fig. 6a–d The bees discriminate sector patterns and transfer as they do for gratings. **a** The positive target has the larger sectors. **b** The trained bees are tested with still larger sectors. **c** The positive target has the smaller sectors. **d** The trained bees are tested with still smaller sectors

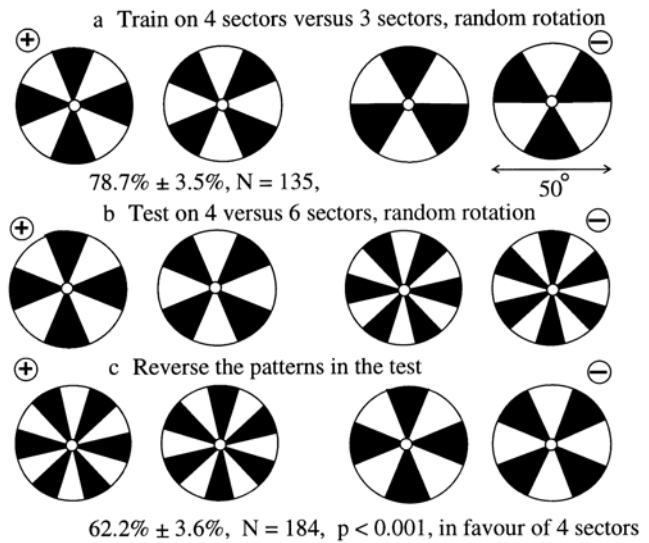


Fig. 7a, b The bees do not rely on the relative number of sectors as a cue. **a** The positive target has more sectors. **b** The trained bees are tested with the previously positive target versus one with even more sectors

that have been trained to a positive pattern that has larger areas of black choose an unfamiliar pattern that has even larger areas of black.

In the converse experiment, a new group of bees was trained on 6 sectors positive versus 4 sectors negative (Fig. 6c). The result after 2 h training was $71.5 \pm 3.2\%$ in favour of the target with 6 sectors. These trained bees were tested 12 times on 6 sectors positive versus 12 sectors negative, and 12 times on 12 sectors positive versus 6 sectors negative (Fig. 6d). The result of the combined tests was $58.3 \pm 3.4\%$ in favour of the target with 6 sectors. They clearly do not recognize the 6 sectors as well as they did in the training combination, as if two memories conflict in the tests, namely, to look for 6 sectors and to choose the target with the larger number of sectors.

A similar experiment was done with targets of fewer sectors. A new group of bees was trained on 4 sectors positive and 3 sectors negative, both with random phase (Fig. 7a). The result after 2 h training was $78.7 \pm 3.5\%$ in favour of the target with 4 sectors. The trained bees were then tested 12 times on 6 sectors positive and 4 sectors negative and 12 times on 4 sectors positive and 6 sectors negative (Fig. 7b). The result of all tests combined was $62.2 \pm 3.6\%$ in favour of 4 sectors. The trained bees had learned well to go to the target with four sectors, but the performance in the tests was reduced as if they had also learned to avoid the pattern with the fewer sectors.

Training on chequerboards with random phase

A chequerboard is a convenient stimulus in that it has a period in two dimensions but the phase can be changed

at intervals so that the bees have no cue but the period. Bees fail to discriminate the 45° rotation of a stationary chequerboard pattern that subtends less than 50° from the point of choice in this apparatus (Horridge 1996b), even with a period of 8.4 cm, so they cannot discriminate the orientations of the separate edges or the locations of the individual areas of black when there are so many.

A group of bees was trained to discriminate between two chequerboards with a change in phase in both every 5 min (Fig. 8a). The positive targets had a period of 9 cm and the negative ones a period of 6.75 cm. The result after 2 h training was 68.5 ± 3.3% in favour of the period of 9 cm. The trained bees were then tested 12 times on period 5.4 cm positive versus 6.75 cm negative (Fig. 8b) and 12 times on period 6.75 cm positive versus 5.4 cm negative (Fig. 8c). The result of all of these tests combined was 61.5 ± 4.1% in favour of the period of 6.75 cm. The trained bees prefer the larger period and not the absolute size of the learned period, as if they have not learned to ignore their innate preference.

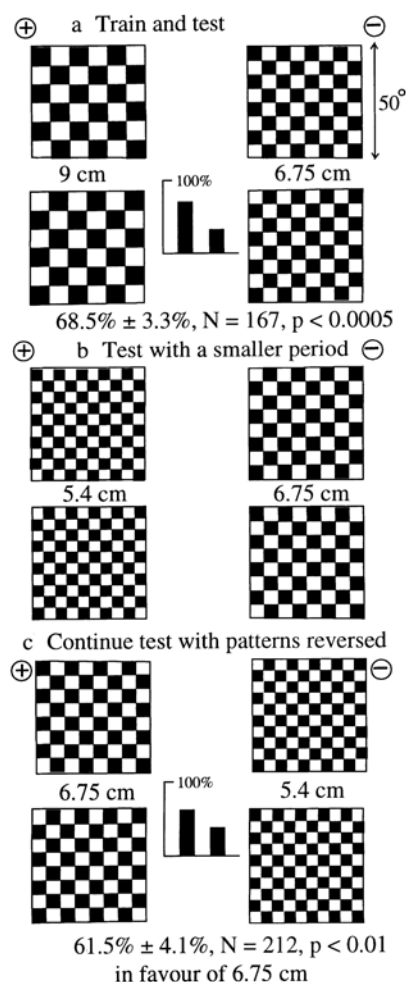


Fig. 8a-c The bees discriminate chequerboards and transfer as before. **a** The positive target has the larger period. **b** The trained bees are tested with the previous negative target versus a smaller period. **c** The test is repeated with the other target rewarded

In the converse experiment (not illustrated), a new group of bees was trained with a period of 6.75 cm positive versus 9 cm negative, with alternating phase as before. After 2 h of training, the performance was 73.8 ± 3.7% ($n = 197$, $P < 0.0001$) in favour of the target with period 6.75 cm. These trained bees were tested 12 times with a period of 6.75 cm positive versus 5.4 cm negative, and 12 times with a period of 5.4 cm positive versus 6.75 cm negative, as in the previous experiment. The result of the combined tests was 57.3 ± 4.4% ($n = 196$, $P < 0.1$) in favour of the target with period 6.75 cm. The performance is poor and the bees act as if they have learned two conflicting cues, to avoid the larger period and to prefer the period of 6.75 cm to which they have been trained.

To test learning of a smaller period, a new group of bees was trained on chequerboards of period 3.9 cm positive versus 5.4 cm negative, and alternating phase every 5 min (Fig. 9a). The result after 2 h of training was 70.9 ± 3.2% in favour of the target with smaller

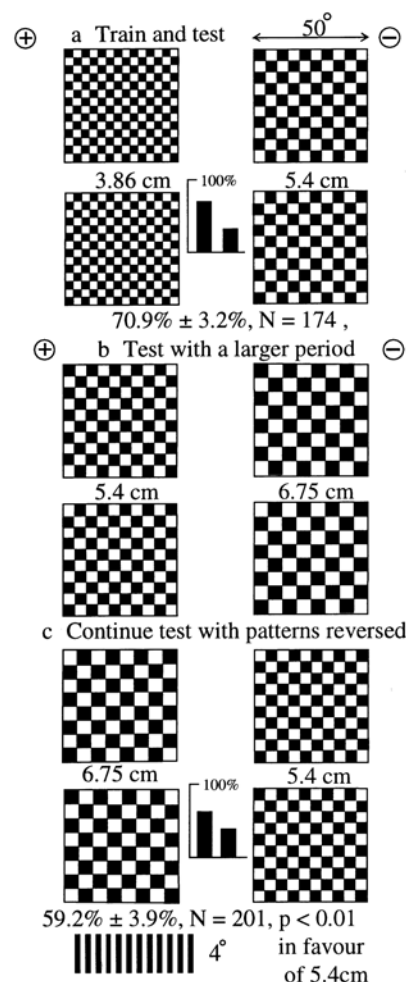


Fig. 9a-c The converse experiment with chequerboards. **a** The positive target has the smaller period. **b** The trained bees are tested with the previous negative target versus one with larger period. **c** The test is repeated with the other target rewarded

squares. These trained bees were tested 12 times on 5.4 cm positive versus 6.75 cm negative (Fig. 9b), and 12 times on 6.75 cm positive versus 5.4 cm negative (Fig. 9c). The result of the combined tests was $59.2 \pm 3.9\%$ in favour of the smaller period of 5.4 cm, but the performance is poor compared to the training situation. The bees behave as if they have learned two cues that conflict in the tests, one to prefer the target with the smaller period, and the other to avoid the previously negative period of 5.4 cm.

Training on spirals with random phase

Spiral patterns are superior to concentric circles as targets, because the phase can be altered at random by rotation without altering the ratio of black to white. A group of bees was trained on a spiral of period 3 cm positive versus one of 4 cm period negative (Fig. 10a). The result after 3 h of training was $76.3 \pm 3.5\%$. The trained bees were tested 12 times with spirals of period 3 cm positive versus period 2 cm, and 12 times with period 2 cm positive versus 3 cm negative (Fig. 10b). The result of all tests was $64.3 \pm 3.4\%$ in favour of the 3-cm period. The bees were able to discriminate the period of 3 cm in the training situation when presented with the period of 4 cm, but the performance is worse when the 3-cm period is tested against a period of 2 cm. Again, this result is as expected if the bees have learned two cues that conflict in the tests, to prefer the period of 3 cm and to avoid the larger of the two periods.

In the converse experiment, a new group of bees was trained for 2 h on a randomly rotated positive spiral of period 4 cm versus a period 3 cm negative (Fig. 10c). The result was $66.6 \pm 3.4\%$ in favour of the positive target (the larger period). The bees were then tested 12 times on a spiral of period 4 cm positive versus one of period 6 cm negative, and 12 times on one of period 6 cm positive versus one of period 4 cm negative (Fig. 10d). The combined result of the tests was $65.8 \pm 3.5\%$ in favour of the broader stripe. In the tests, the bees fail to recognize the previously positive period of 4 cm when presented in combination with one of 6 cm. Instead, they prefer the pattern with the larger period, as happened with the other types of pattern.

A gradient of spatial frequency

An array of regular units such as triangles, squares or discs can form a gradient of disruption with large elements at one end and small ones at the other (Fig. 11). If the units are of constant shape, orientation, and relative separation, the positive pattern can be identical to the negative pattern with the gradient, but not the triangles, rotated through 180° . In the examples shown here, the patterns contain 50% black in every region.

With a graded array of triangles, the positive target with large triangles at the top, the negative one with

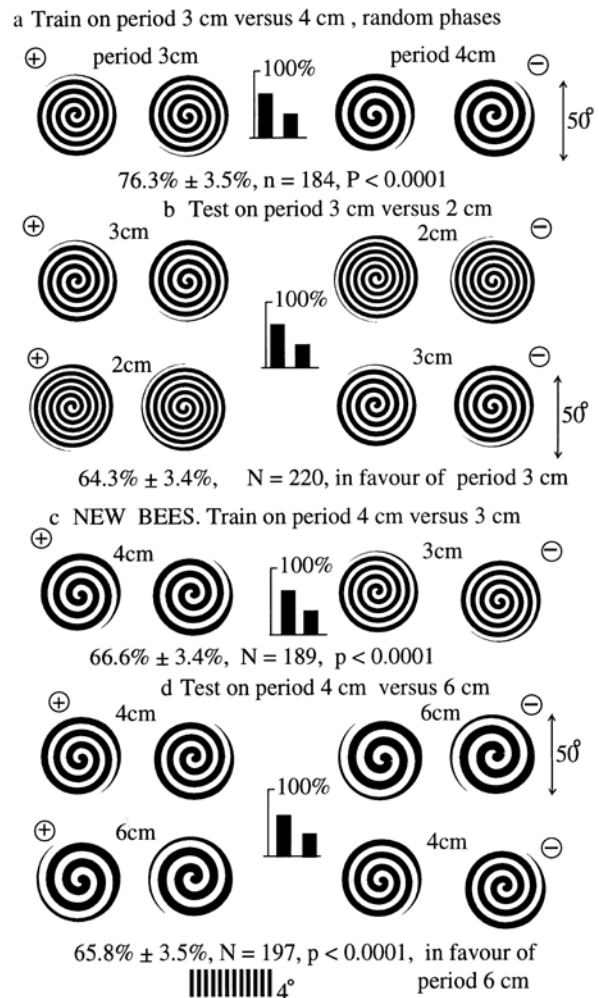


Fig. 10a–d The bees discriminate spirals and transfer as before. **a** The positive target has the smaller period. **b** The trained bees are tested with the previous positive target versus an even smaller period. **c** The positive target now has the larger period. **d** The bees trained in **c** are tested with the previous positive target versus an even larger period. As with gratings and checks, they prefer the larger

large triangles at the bottom (but all black triangles pointing down), the bees cannot discriminate the two targets at 27 cm from the baffle (Fig. 11a). The period of the array (centre-to-centre of the black triangles) ranged from 3.7 cm at the big end to 1.3 cm at the small end. The result was $49.1 \pm 3.7\%$. The bees spend a long time looking at the targets and never learn, however long the training, although the locations of the areas of black are fixed. With a new group of bees, and the targets at 9 cm from the baffles and subtending 100° at the point of choice, the situation was quite different. The result was now $68.9 \pm 3.5\%$ ($n = 151$) after only 1 h of training, in agreement with previous findings that fixed areas of black act as landmarks in very large targets (Horridge 1996b).

A new group of bees was trained with a similar graded array, but this time of circular discs separated by the appropriate distance to make the pattern 50% black

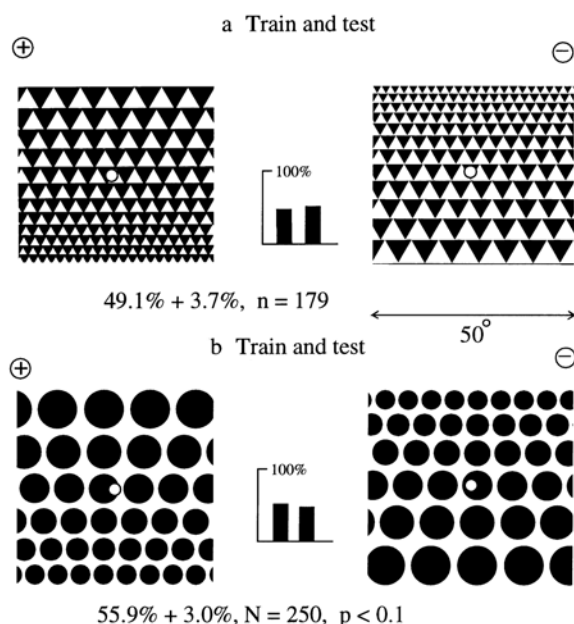


Fig. 11a, b Bees fail to discriminate between two patterns of regularly repeated elements with different spatial distributions of the unit size. **a** triangles. **b** discs

in all regions. The positive pattern had large discs at the top; the negative one had small discs at the top (Fig. 11b). The discs in (b) are larger than the triangles in (a). With the targets 27 cm from the baffles, the performance was $55.9 \pm 3.0\%$, which is indicative though not significant. This result suggests that the locations of the largest areas of black might be discriminated because the patterns are fixed.

With the same targets at 9 cm from the baffles and a new group of bees, the locations of the discs relative to the reward hole were randomized by taking four varieties of the pattern, with differently located reward holes, in turn. With this arrangement, although the pattern subtends 100° at the point of choice, the bees cannot discriminate the gradient in the pattern from that in the same pattern turned upside down, presumably because the contrasts are no longer in fixed locations.

The failure of the bees to discriminate these graded patterns supports the view that, apart from remembering the location of large constant areas of black, the bees can make use of only a limited variety of cues. The patterns in each pair in Fig. 11 offer no global average difference in disruption, or any other known cue that is of use to bees, although they are obviously different to human vision.

Discussion

The randomization technique to make the locations of areas of black useless as cues distinguishes these experiments from previous work on the disruption of the pattern as a cue. In the horizontal presentation used by

Hertz and by Zerrahn 60 years ago, and sometimes by Anderson, the patterns were shuffled at intervals on a flat table so that the locations of black areas were useless as cues. Differences in pattern disruption, pattern size and radially symmetrical differences were the only remaining cues, as indeed Hertz proposed. In addition, the bees acted as if unable to correlate the orientations of edges with the directions of their own flight lines, so edge orientation was also useless as a cue. However, the criterion was the landing of the bees on the target so it was also impossible to define the angular spatial frequency of the cue at the eye of the bee. When patterns were presented on a vertical plane and the bees made to choose from a distance, it became evident that they could learn the locations of areas of black (review: Wehner 1981). As a result of this finding, nothing can be said about the discrimination of disruption in patterns presented vertically unless the learning of fixed locations is ruled out. In the present work, the pattern phase was switched every 5 min, so the bees were actually trained to ignore the locations of areas of black. This measure left the bees with only one cue that might be used to discriminate the positive patterns from the negative ones.

Recently it has been shown that flying bees have an innate preference for larger periods when given a forced choice between a number of patterns from a distance, and this is true for a wide range of patterns (Lehrer et al. 1995). This finding is important for the interpretation of the present results but it conflicts with the earlier findings of Zerrahn (1933) and Anderson (1977) that bees spontaneously prefer patterns that are more disrupted. The conflict could arise in three ways.

First, in their work, the bees were counted as they landed on the pattern, and bees already feeding were visible on the patterns. As can be seen on our bee feeders every day, the arriving bees are strongly attracted to the bees that have already landed. The most preferred patterns in Zerrahn's work had black bars or spots about the size of bees and could have resembled a group of landed bees. Hertz (1933) says that her method should be improved by using marked bees arriving one at a time and disappearing into the reward box, as in the experiments of von Frisch (1914), so that the successful bees did not attract other bees.

Secondly, disruption on a fine scale appears to be the signal for landing near the centre of a flower. Anderson's most attractive striped patterns had a period of only 1 mm, which would become resolvable only when the bee approached within about 15 mm, which is a far cry from the patterns viewed by bees at a distance. Anderson found that his most attractive fine lines had similar dimensions to those of nectar guides at the centres of many flowers, so, when the criterion for choice is the landing of the bee, the experiment reveals where the bees prefer to land, and not the pattern disruption that the eye prefers at a distance. Many experiments with artificial flowers show that nectar guides have no influence on pattern preferences at a distance (Manning 1956; Dau-

mer 1958), and, indeed, the fine lines used by Anderson would be below the resolution limit of the eye.

Thirdly, in the Y-choice apparatus, the pattern with the largest period is simply the one that is resolved first by the approaching bee, and which appears to be the nearest, whereas bees that scan a surface and then land on it have an unknown selection of cues.

No matter whether the pattern is a regular grating, radial sectors, chequerboards or spirals, the bees rapidly learn a difference in period, although the locations of black areas are regularly shuffled. In these experiments they easily remember a difference of 25%. Apart from the magnification, the two patterns in each choice are identical, so there are no different average intensities or orientations of edges for the bees to discriminate. The ability to discriminate when the patterns are changed to quite different patterns implies that the bees can use a relative feature related to the disruption. This could be the period, the modulation at the eye, the total length of edge, the ratio of edge to area, or whether to avoid or prefer the target carrying the single largest area of black. Within the idea of disruption, there are several possible generalized cues.

When tested with similar patterns at other scales, trained bees can be made to accept the previously negative target, so revealing that they have not learnt to prefer the particular period of the positive target in isolation. In fact, to learn an absolute value of a spatial frequency would not be an effective strategy for a flying insect choosing between two objects or fields that vary independently in range, but in special circumstances it could be an effective way to recognize an object or target in the natural world if the bee hovers always at the same range. The angular spatial frequency of a target at the bees eye is inversely proportional to range, but it would be possible to have a set range at which a discrimination is made. Observations of freely flying bees might reveal this. Also, a relative difference in disruption might be a useful cue about the general nature of the surrounding scene when combined with information from landmarks.

The innate preference for the larger period means that when training reinforces an existing tendency, the bees do not necessarily learn much, because they have the opportunity to learn only when they make errors. Recently, Ronacher (1992) found that bees trained to prefer the larger of two shapes prefer a still larger shape, which suggests that the innate preference has not been over-ruled in the learning process. Therefore, the experiments in which the bees had to learn the smaller rather than the larger period are more suitable for evaluating the bees' performance.

When trained to go to the smaller of two periods and then offered a forced choice between the positive period and a still smaller period, the trained bees prefer the previously positive period rather than a still smaller period (Fig. 3). When trained to go to a small period and then offered a choice between two larger periods, the bees select the smaller of the offered periods but perform badly if it was the previous negative pattern (Fig. 9).

These preferences persist irrespective of a change in the type of pattern in the tests.

Three general rules emerge from the results: first, when the positive pattern has the larger period, the bees choose as if they use their innate preference for larger periods as seen from a distance (Figs. 4, 5, 6, 8). When the positive pattern has a smaller period, the bees are obliged to overcome their innate preference, which they do very well. They do not transfer their preference for the smaller period to a still smaller period (Figs. 3, 7, 10b), showing that they do not discriminate between other periods accordingly to whether they are the larger or smaller. Apparently, as one cue, they learn to avoid the larger period of the negative pattern and, as another cue, something about the period of the positive pattern itself. Perhaps this is the size of the unit, the disruption or some other feature related to spatial frequency. The bees are confused when these two cues contradict each other in tests (Figs. 3c, 6d, 7, 10b). Secondly, the results of forced choices show that the bees do not rely on learning a particular angular spatial frequency subtended at the eye. Thirdly, the results hold for a variety of patterns and trained bees are not greatly disturbed by a change in pattern, as if they use a global feature as a cue and care nothing for the actual pattern.

As illustrated in Fig. 2, bees can discriminate between pairs of patterns by the different locations of areas of black. If locations of areas are randomized, bees are still able to use a variety of generalized global attributes of the image, but there is no experimental evidence that they reconstruct the relative spatial lay-out of the individual local features. For example, they fail to discriminate a fixed triangle from the same pattern turned upside down (Fig. 2a). Bees do not remember the locations or orientations of the individual bars in fixed patterns subtending 50° at the eye, when the patterns consist of orthogonal pairs of four equal bars, but the same patterns can be discriminated if they differ globally in radial or circular components (Horridge 1996a) or in the axis of bilateral symmetry, irrespective of pattern (Horridge 1996c). Although the part of the bee visual system that controls locomotion has to deal all the time with complex natural scenes containing many shapes at all scales, the confusion of simple shapes by bees strongly suggests that their discrimination system does not re-assemble the lay-out of local features of the image, such as blobs and edges, in their relative spatial locations.

Numerous tests with different types of patterns show that when the locations of contrasts are made useless as cues, bees can be trained to a generalized feature, such as range (Lehrer et al. 1988), orientation (Srinivasan 1994), radial and circular features (Horridge and Zhang 1995), target size (Horridge et al. 1992), bilateral symmetry about an axis (Horridge 1996c), and now disruption. The bees behave as if they remember only the global cues, the actual patterns being irrelevant. So far, the only data on the discrimination of different textures, or of the same texture at different orientations and scales, suggest

that bees do not re-assemble the texture, but merely extract and remember a few global cues.

The theory that bees distinguish between objects visually by the amount of flicker that they generate as the eye moves, has enjoyed a wide acceptance for several reasons in no way related to the decisiveness of the original experiments. Until Anderson (1972, 1977), there were no tests on whether the bees could in fact discriminate different amounts of disruption when presented in the same pattern. The disruption theory has had a long life because it was the only one that included a generalized filter for a feature, irrespective of the pattern. As alternatives, there were the vague holistic terms such as “figural quality” derived from Gestalt theory of human vision as used by Hertz (1933), and the memory of location in very large targets, as discovered by experimenting with fixed vertical patterns (Wehner and Lindauer 1966). A photograph of locations on the retina, however, is not a theory of visual processing, which requires an indication that the separate responses of the photoreceptors are correlated. Apart from the orientation of long edges in very large targets, no other cues were revealed until the use of randomized locations of areas of black in the present decade.

What then is the function of the discrimination of the average disruption of the visual field that is so easily demonstrated? Perhaps knowing the spatial frequency is an essential step in the visual measurement of the velocity in flight, or it may be a method of discriminating between natural scenes. An important visual task for the bee is route finding and recognition of the various scenes in its territory. Such a memory would enable the bee to tag or code a simple summary of each large area in the visual field, like bushes, trees, plants of differing leaf size, grass, a wall, water surface, and so on, without committing any detail of the scene to memory. Perhaps this is all that a small brain can manage, and this global, as opposed to local, cue has the advantage that single edges and many parts of natural scenes behave like fractals in that they do not change their overall distribution of spatial frequencies with range, but can be distinguished from each other at any range. For a bee in flight, the average disruption in a visual field could be a single parameter that would mark a sudden change in the surroundings, distinguish a particular habitat, or discriminate between two scenes.

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