

Generalization of convex shapes by bees: what are shapes made of?

Miriam Lehrer^{1,*} and Raymond Campan²

¹*Department of Neurobiology, Institute of Zoology, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland* and ²*Laboratoire d'Ethologie et Cognition Animale, Université Paul Sabatier, 118 route de Narbonne, F-31062 Toulouse, France*

*Author for correspondence (e-mail: miriam@zool.unizh.ch)

Accepted 11 July 2005

Summary

For about 70 years, bees were assumed not to possess the capacity to discriminate among convex shapes, such as a disc, a square or a triangle, based on results of early studies conducted by presenting shapes on horizontal planes. Using shapes presented on a vertical plane, we recently demonstrated that bees do discriminate among a variety of convex shapes. Several findings, summarized here, provide indirect evidence that discrimination is based on a cue located at the shapes' boundaries. In the present study, we test this hypothesis directly in two different ways. (1) Three groups of bees are each trained with a different pair of convex shapes, one positive (rewarding), the other not (negative), producing colour contrast, luminance contrast or motion contrast against the background. The trained bees are then offered a choice between pairs of stimuli whose shapes are identical to those of the training shapes, but whose contrast against the background is varied by changing the pattern, the colour or the luminance of the areas. The results show that

bees discriminate between the pairs of novel shapes, i.e. they generalize the shapes among the different types of contrast, revealing that they use a particular cue extracted from the positive shape. The bees' choices between a stimulus that produces the correct contrast but has the wrong shape and one that possesses the correct shape but the wrong contrast show, in addition, that the relevant cue is not located within the area of the shape. (2) Bees trained with pairs of convex shapes are tested with the same pairs of shapes, but which lack the inner area, i.e. only the contours or fragments of the contours are presented in the tests. Bees are found to prefer the stimulus whose contours (or fragments of contours) agree with those of the positive training shape. Taken together, the results suggest that convex shapes are not represented by the form of their areas but rather by some cue located at their boundaries.

Key words: honeybee, shape discrimination, convex shape, colour contrast, luminance contrast, motion contrast, generalization.

Introduction

Discrimination among convex shapes is one of many aspects of spatial vision that has been investigated in the bee. A convex shape (also termed 'closed shape'), such as a disc, a triangle or a square, is defined as a geometrical figure in which every line connecting two points on the circumference lies within the area of the pattern. Shapes that do not meet this condition, such as star shapes, gratings or checkerboards, are termed 'disrupted shapes'. In human vision, a triangle and a square are clearly two distinct shapes that humans categorize regardless of their size, colour or alignment in space. As opposed to this, in bee vision, discrimination of convex shapes constitutes a special problem that behavioural studies have not solved satisfactorily until the present day.

The older studies, conducted using patterns presented on a horizontal plane, denied the bees the capacity of discrimination between convex shapes (e.g. von Frisch, 1915; Baumgärtner, 1928; Hertz, 1929, 1930, 1933; Zerrahn, 1934; Wolf and Zerrahn-Wolf, 1935; Manning, 1957; Autrum, 1957). They showed repeatedly that the most effective discrimination

parameter was the spatial frequency of the pattern, i.e. the amount of contours per unit area, also termed 'pattern disruption'. Indeed, convex shapes do not differ much in their (very low) spatial frequency, which was considered a good explanation of the bee's failure to discriminate among them. More recent studies, initiated by Wehner and Lindauer (1966), were conducted using patterns placed on vertical planes. Even with this mode of presentation, spatial frequency was shown to constitute a powerful spatial parameter (Wehner, 1971, 1981; Anderson, 1977; Horridge, 1997; Lehrer, 1997). However, several further parameters were found to be effective as well: size (Ronacher, 1979; Wehner, 1981; Horridge et al., 1992), the presence or absence of corners (e.g. disc vs triangle; Ronacher, 1992), the number of corners if present (Mazokhin-Porshnyakov, 1969), brightness or contrast (Ronacher, 1979, 1992; Lehrer and Bischof, 1995; Ronacher and Duft, 1996; Hempel de Ibarra and Giurfa, 2000), the spatial orientation of contours (Wehner, 1971; van Hateren et al., 1990; Srinivasan, 1994; Horridge, 2000), symmetry (Lehrer et al., 1995; Giurfa

et al., 1996a; Horridge, 1996; Lehrer, 1999a) and even the centre of gravity of the pattern (Horridge, 2003; for a similar finding in the fruit fly, see Ernst and Heisenberg, 1999). The use of a particular cue for shape discrimination became known as the 'feature extraction theory', or the 'parameter hypothesis'. According to this theory, the animal extracts and memorizes a particular cue out of several possible cues that are present in the rewarding stimulus. The learned cue is recognized in novel stimuli even if they disagree with the rewarding one in all other parameters. The transfer of visual information acquired in a particular situation to another situation is termed 'abstraction' or, more commonly, 'generalization'. Generalization is typical of every process that involves feature extraction. The generalization of a particular cue therefore serves as a reliable criterion to establish whether or not discrimination is based on feature extraction.

Results obtained using the vertical mode of presentation have, however, given rise to an alternative theory, termed the 'template theory', or the 'image-matching hypothesis' (Wehner, 1972a,b, 1981; Cruse, 1972; Collett and Cartwright, 1983; Gould, 1985; Ronacher and Duft, 1996; Collett, 1996; Collett and Zeil, 1997). It proposes that, during training, bees learn the retinal positions of contrasting areas contained in the image of the rewarding pattern (or in a given constellation of landmarks). The distribution of contrasting areas is stored eye-region specifically, as a retinotopic template. Shape discrimination is then accomplished by comparing the currently viewed image with the learned one. Discrimination is better, the larger the discrepancy of the retinal positions of contrasting areas between the two images. Retinotopic learning is only possible when the spatial relations between pattern and eye are kept constant. This condition is only met when the stimuli are presented on vertical planes, and are therefore approached frontally, and when they contain, at the same time, a conspicuous site that bees are able to use as a fixation point prior to landing (see, for example, Wehner and Flatt, 1977). Image matching is not possible when patterns are presented on horizontal planes, where there is no fixation point and when bees approach the target from all possible directions.

Thus, in the vertical plane, both image matching and feature extraction are possible, so that it is often difficult to decide whether the former or the latter is responsible for an observed discrimination performance. For example, when a half-white/half-black disc trained in a particular angular orientation is tested against a disc that has been rotated in small steps relative to the learned disc, bees discriminate the two discs better, the larger the amount of rotation (Wehner, 1972b). One explanation of this result would be that bees have learned the orientation of the edge between the two halves of the disc. The other would be that the black area overlaps less with its memorized position the more the disc is rotated. Similar considerations hold true for coloured half-discs (Menzel and Lieke, 1983; Lehrer, 1999b) and even for spatial parameters other than edge orientation (Efler and Ronacher, 2000). Bees can, however, be prevented from using image matching by randomizing the distribution of contrasting areas during the

training, keeping the parameter under consideration, for example the distance of the target from the flight plane (Lehrer et al., 1988) or the orientation of contours (van Hateren et al., 1990; Srinivasan, 1994; Horridge, 1997), constant.

Interestingly, systematic studies on bees' capacity to discriminate among convex shapes have never been repeated using the vertical mode of presentation. The idea that bees do not possess this capacity has been so widely accepted that convex shapes were hardly ever used in studies on shape discrimination for about 70 years. Of course, although without experimental evidence, the lack of discrimination could be explained even under the image matching theory: convex shapes, provided that they do not differ in size, display only small amounts of non-overlapping areas (see, for example, fig. 4 in Campan and Lehrer, 2002). Thus, neither the parameter extraction theory nor the image matching theory provided reasons to believe that bees discriminate among convex shapes.

It was not before 2001 that new results demonstrated that bees do discriminate among convex shapes (Lehrer and Campan, 2001; Campan and Lehrer, 2002). We used five different convex shapes of equal areas (100 cm²) presented on a vertical plane, training five groups of bees each to one of the five shapes in turn. In each experiment, the trained bees had to choose between the learned shape and each of the four other shapes. Bees accomplished the discrimination in all five training experiments, comprising 20 different tests. Although not all pairs of shapes were discriminated equally well, the proportions of correct choices were not correlated with the degree of overlap among contrasting areas, from which we concluded that discrimination was not based on the comparison of areas. The only alternative possibility was a cue that is located at the circumference of the shapes (Campan and Lehrer, 2002), which is the site where the shape produces contrast against the background.

There are three different types of contrast that render an object visible, provided that contrast is above resolution threshold: colour contrast, luminance (intensity) contrast and motion contrast. In all cases, the larger the amount of contrast, the better the visibility of the object.

Colour contrast has been shown to participate in spatial vision (e.g. Menzel and Lieke, 1983; Ronacher, 1992; Lehrer, 1999b) and to enable object detection and shape discrimination even in the complete absence of luminance contrast (Menzel and Lieke, 1983; Lehrer and Bischof, 1995; Giurfa et al., 1997). Whenever two stimuli differ in colour, they produce colour contrast against each other. The highest colour contrast is produced by complementary colours.

Although colour perception is independent of light intensity, colour contrast is often accompanied by luminance contrast, whereas luminance contrast never includes colour contrast. The highest intensity contrast is produced by black-and-white stimuli. The luminance perceived is a result of either the summed response of all three spectral types of receptor or only of one of them exclusively (for example, the green-sensitive receptor; see Discussion). In either case, the contrast is

achromatic, because colour cannot be computed unless the responses of the three spectral types of receptor are each determined individually (see, for example, Backhaus and Menzel, 1987 and excellent illustrations in Menzel, 1987).

The third type of contrast, motion contrast, is perceived either when a patterned object moves against a patterned background or else when the observer moves in front of an object that is placed at some distance from a patterned background. In the latter case, both the object and the background move at the observer's eye, but the object moves faster than the background, because it is nearer. The bees' use of motion contrast (motion parallax) for object detection has been demonstrated by Srinivasan et al. (1990), who found that detection of a patterned figure placed above a similarly patterned background improved with the height of the figure above the background and thus with the amount of motion parallax that it produces.

Pure motion contrast, i.e. one that is not accompanied by colour contrast or luminance contrast, is produced when both the shape and the background display the same pattern, the same colour and the same intensity. In this case, motion parallax is the only cue that renders the shape visible. There exists, however, another type of motion-induced contrast between figure and background, examined quite extensively in pigeons (Frost et al., 1990), termed 'covering parallax'. It occurs when a homogeneous object is presented at some distance from a patterned background. In this case, the area of the object does not move at the eye, but its contours move across the background, the leading contour covering the pattern, the trailing contour uncovering it. Covering parallax always involves pattern contrast, because the homogeneous object displays no pattern whereas the background does. The inverse combination, i.e. patterned shapes presented against a homogeneous background, also contains pattern contrast, but it produces neither motion contrast nor covering parallax.

Our conjecture that the cue used in the discrimination of convex shapes is located at the site where contrast occurs (Campan and Lehrer, 2002) received strong support from results obtained in the same study using randomly patterned black-and-white shapes presented in front of a similarly patterned background. An earlier study (Srinivasan et al., 1990) has already shown that such shapes are invisible to the bees when they are placed directly on the background, even when they carry a much coarser pattern than the background. However, because our shapes were placed at some distance from the background (see Fig. 1A), they move faster at the bee's eye, thus producing relative motion (motion contrast) visible at the boundaries between shapes and background. Discrimination was excellent in all 20 tests, although here the bees could not rely on the areas of the shapes (Campan and Lehrer, 2002). Furthermore, in an earlier study by Ronacher and Duft (1996), bees trained to discriminate between a black triangle and a delineated disc accomplished the discrimination even when a delineated triangle was tested *versus* a black disc. More evidence came from a recent study

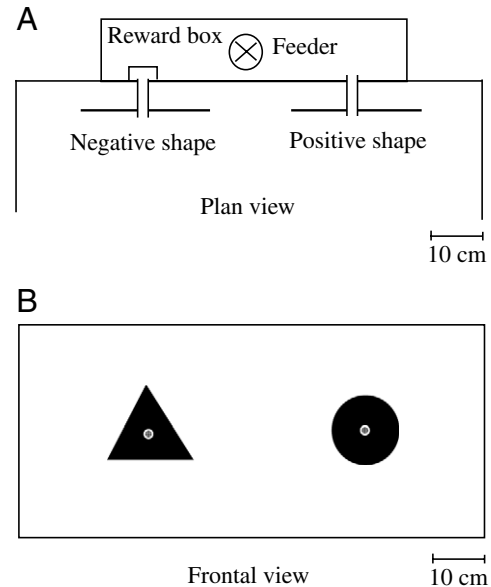


Fig. 1. The experimental apparatus. (A) Plan view; (B) frontal view. Two shapes, one rewarding (termed positive), the other not (negative), are presented simultaneously, each mounted on a 50 mm-long Plexiglas tube that penetrates its centre and connects the front wall of the apparatus to the reward box fixed behind it. The tube associated with the positive shape is open (A, right) whereas the other ends blindly. After training bees with a particular pair of shapes, they are tested by offering them a choice between the positive shape and each of several novel shapes (discrimination tests) or between a novel shape and other novel shapes (transfer tests), as explained in the text. The first choice of each bee on each of its visits is recorded. Choice frequency (CF), i.e. the percentage of choices for a given shape, is calculated from the sum of choices (N) obtained in each type of test.

by Hempel de Ibarra and Giurfa (2003) using a pair of coloured convex shapes (a triangle and a square) that produced different amounts of colour contrast and luminance contrast against a homogeneous grey background. The authors found that discrimination requires the presence of green contrast, which is achromatic (see above). This finding agrees well with some of our earlier results showing that following of contours (Lehrer et al., 1985) and edge detection (Lehrer et al., 1990) is under the control of the green-sensitive receptors. Thus, discrimination of convex shapes seems to be closely related to edge detection.

The present study is an attempt to provide more direct evidence for the role of edges in the discrimination of convex shapes. In our experiments, we train bees to discriminate between two convex shapes, using a different pair of shapes and fresh bees in each experiment. The two training shapes produce the same type of contrast against the background, but the type of contrast is varied by varying the pattern, colour or luminance of the area of the shape, or of the background. Changing the appearance of the area does not change the parameters of the contours, i.e. their position, or alignment, their number, or their length. If bees trained with one type of contrast accomplish the discrimination even with novel types of contrast, then this might

be due to one of two different reasons. First, bees might have learned and generalized the shape of the area regardless of its appearance (colour, pattern), i.e. shape perception is independent of the type of contrast. The alternative explanation would be that the shape of the area and therefore its appearance is unimportant because the relevant cue is not located in the area but rather at the circumference of the shape. It should be possible to discriminate between the two possibilities by placing edges and areas in competition. If one of the two cues is more effective than the other, then bees are expected to prefer it.

Based on these considerations, we conducted two sets of training experiments. In the first set, three experiments (Expts 1–3) were conducted in which we investigated (1) the generalization of shape among different types of contrast and (2) the bees' preferences between the correct shape and the correct contrast. Of course, control tests had to be conducted as well to establish that the test shapes were indeed novel to the bees, by showing that bees discriminate the preferred test shape from the shape that they have learned during the training.

In the second set of experiments, four groups of bees were trained using two pairs of patterned convex shapes presented in front of a patterned background. Each pair of shapes was trained reciprocally with a fresh group of bees. The trained bees were then given a choice between pairs of shapes that were identical to the training shapes but displayed only the circumference of the shapes or only fragments of it. If bees use the edges in the discrimination task, then they are expected to accomplish the discrimination even when the shapes possess no inner area. Again, as a control, tests were conducted to show that the stimulus consisting of contours alone is discriminated from the complete shape used during the training.

Materials and methods

The experiments were conducted on local races of honeybees (*Apis mellifera* L.) in the summers of 2003 and 2004, some in a private garden in a suburb of Zurich (Switzerland) and some in a private park in the suburb of Toulouse (France). The method employed in Toulouse differed from that employed in Zurich in only a few small details, which will be pointed out.

In Zurich, bees that happened to forage in the garden were trained to visit a permanent feeding dish containing sucrose solution placed on a low table about 3 m away from the experimental apparatus. Bees arrived from the north from a hive that was unknown to us. They required, on average, 5.6 ± 1.7 min between visits (19 measurements on eight bees). This time interval included the time spent feeding at the dish and unloading at the hive. From the approximate net flight time, we calculated that the hive was located ~1 km away from the experimental site.

In Toulouse, we had our own hive in the park, located in a south-westerly direction ~500 m away from the experimental site. In Zurich, as well as in Toulouse, the permanent feeding place was maintained throughout the summer to serve three functions. First, it kept newcomer bees recruited to the

experimental site away from the apparatus, because the open feeder was easier to find and more comfortable to feed from than the feeder within the apparatus (see below). Second, whenever a fresh group of bees was needed for a new training experiment, they could be trained without delay from the very near feeder to enter the apparatus. Finally, the permanent feeder ensured that the experimental bees kept returning to feed, even early in the morning and late in the afternoon when the apparatus was closed. As soon as the experiment was resumed, they resumed their visits to the apparatus, where the concentration of sucrose solution was kept higher than that at the permanent feeding place.

Experimental apparatus

The experimental apparatus (Fig. 1) was similar to that used in our earlier study (Campan and Lehrer, 2002). It was placed outdoors under a roof, thus being protected from rain and direct sunshine. In Toulouse, the apparatus stood free but it had a translucent roof of its own to protect against rain. Depending on the experiment, the front wall, which served as the background against which the shapes to be used were presented, was covered either by homogeneous white paper or by a black-and-white random pattern produced on the computer by printing black squares (0.5×0.5 mm² in area) on the same type of white paper. The white paper had a flat spectral reflection curve (see fig. 2 in Lehrer and Bischof, 1995); i.e. it was achromatic to the bees.

Two Plexiglas tubes, 40 cm apart and 18 mm in diameter (30 mm in Toulouse), were inserted in the front wall of the apparatus, connecting it with a dark reward box affixed behind the wall. The box contained a small feeding dish with sucrose solution placed in the middle between the two entrances. The tubes were 60 mm long, so that after having inserted their ends in the reward box they protruded by 50 mm out of the front wall. The shapes to be discriminated, each with a suitable hole in the centre, were mounted on the front end of each tube and thus at a distance of 50 mm from the background. One of the shapes, termed positive, was rewarding; the other was not (negative). Bees could enter the reward box through either of the two tubes, but only the one that carried the positive shape gave access to the food, because the other tube, carrying the negative shape, ended in a small closed box from which the bees could not reach the feeder. Bees that had entered through the blind tube had to exit and try again. This device constituted a kind of punishment for bees that made a wrong choice, because they often struggled for up to 35 s to reach the feeding dish from the closed box before they turned around to exit. Thus, bees were encouraged to learn not only which shape was rewarding but also which shape should be avoided. The two shapes interchanged their places after every two to three rewarded visits (on average for all bees) to prevent the bees from developing a preference for the right or the left tube.

In Toulouse, each tube had a reward box of its own, but only the box associated with the rewarding shape contained a feeder with sucrose solution. Both tubes were open, but the entrance to the reward box had a one-way flap. A bee that had entered

the empty box could not return through the tube and was forced to leave the box from behind through another one-way flap. It was then free to fly around the apparatus and approach from the front to make a new decision.

Initiating a training experiment

Prior to each experiment, bees feeding at the permanent feeder were transported passively, along with the feeding dish, into the dark reward box. Upon departure, most bees performed the so-called 'turn-back-and-look' behaviour (TBL; Lehrer, 1991, 1993), turning around to take a close look at the apparatus before leaving for the hive. These bees soon returned to the experimental site, where they eventually found the entrance to the reward box. After their first active arrival, the bees were marked individually with coloured spots on thorax and abdomen whilst they were feeding. For each experiment, 12–15 bees were marked and trained.

As already mentioned above, the concentration of the sugar solution offered in the reward box was kept higher than that offered at the permanent feeding place, to make the experimental bees take the trouble to creep through the tube. The trained bees switched to the permanent feeder only when the experiment was interrupted, in which case the apparatus was covered with a large dark blanket. They clearly learned the difference in food quality: as soon as the apparatus was exposed again, they left the permanent feeder in a great hurry and rushed at the tube entrances.

Scoring

On each visit, the bees' choice between the two shapes was recorded. A choice was defined as entering the tube associated with one of the shapes. A choice in favour of the positive shape scored a plus point, and a choice for the negative shape a minus. On every arrival, only the first choice of each bee was recorded, i.e. entering the tube at the positive shape scored a plus only if the bee had not entered the negative tube before landing on the positive one. Bees that had made a mistake usually corrected immediately by flying straight to the positive shape after having left the wrong tube. Had this second choice been recorded in addition, then that bee would have scored one minus and one plus point, whereas a bee that entered the correct tube on its first trial receives one plus. This would render 66.7% correct choices, although the true proportion was only 50%. Furthermore, our scoring method ensured that each of the individual bee's choices was independent of its previous choice. In Toulouse, where bees were forced to leave, but were able to return and try again, a bee's first decision after resuming its search was recorded again. No bias in favour of the positive shape was found using this method.

For each test, the proportion of plus points [choice frequency (CF)] was calculated from the total number of choices recorded in that test.

Training

The two shapes to be used in the training were mounted on the two tubes prior to each experiment, so the bees saw them

as soon as they performed the first TBL. In each experiment, the two shapes used during the training were kept constant throughout the experiment. For quantifying the success of the training, the bees' choices during the training were recorded from the very beginning of the training. Recording for periods of 10 min every hour on the first day, and at least twice every day on the following days, informed us about the level of learning. The final CF in favour of the positive training shape was calculated from all the choices recorded during the training throughout the experiment, with the exception of the first hour, which always rendered the poorest result.

Discrimination tests and transfer tests

Two different types of test were conducted with the trained bees. In the first type of test, termed discrimination tests, the positive test shape was identical to the positive training shape, whereas the alternative shape (the negative test shape) was novel to the bees. In the second type of test, termed transfer tests, both shapes (or sometimes the background) had a different colour or a different pattern from during the training. The type of contrast encountered in the transfer tests was thus always novel to bees. However, the positive stimulus had the same shape as the positive training shape, whereas the alternative stimulus had either the same shape as the negative training shape or a different, novel shape. During the tests, bees continued to be rewarded on the stimulus that had the learned shape. Reinforcement during the tests ensures that the bees neither accumulate in the apparatus during the test nor learn to avoid the apparatus whenever they notice a novel situation. In earlier studies, it has already been demonstrated that short periods of reward on novel shapes do not interfere with the memory of the learned shape even when the test shapes differ much more from the training shape than is the case in the present study (see, for example, Van Hateren et al., 1990; Srinivasan et al., 1994; Horridge, 1997). Even in experiments comprising dozens of tests, results of tests conducted at the end of an experiment proved to be very similar to those obtained with the same shapes at the beginning of the experiment (Campan and Lehrer, 2002). In the present discrimination tests, the positive test shape was, in any case, identical to the positive training shape, and in the transfer tests, the rewarding shape still had the form of the positive training shape, meaning that at no time were the bees made to learn a new shape.

Each test lasted for a total of 15–20 min, the positive and the negative test shapes being presented on one of the tubes for half of the time and on the other tube for the other half. After each test, training with the two original training shapes was resumed for at least 30 min. If a test had not rendered at least $N=100$ decisions within the maximum of 20 min, it was repeated on one of the subsequent days until the total number of decisions was at least 100.

Control tests

A further two types of test served as controls. One served to examine whether or not the results of the transfer tests are based on a true generalization performance. In these tests, the

shape preferred in the transfer test was presented against the positive training shape. Generalization can only be inferred if the two shapes are discriminated, i.e. if the preferred test shape is indeed novel to the bees.

The second type of control test served to ensure that the bees' choice behaviour was based on shape recognition and not on having learnt to discriminate between the open and the plugged tube. In these tests, the open and the blocked tube carried identical shapes. If bees choose randomly between them, then we may conclude that they cannot tell the open tube from the blocked one, i.e. discrimination is based exclusively on shape recognition.

Statistics

Tests for the significance of the differences between the results obtained in the tests and the values expected under random-choice conditions ($P=Q=0.5$) were conducted using the χ^2 test. Throughout, these tests were only performed in cases in which the significance or non-significance was not clear at first sight. For example, at the 5% significance level, if $N \geq 100$, then $CF \geq 60$ always renders significance against the random-choice expectation. Similarly, when comparing between results obtained in two independent experiments, not every pair of results required a test for significance of the differences. We mainly compared complete series of results, using the Wilcoxon signed ranks test.

Experiments and results

Expt 1. Colour contrast: training with a pair of figures that differ in colour but not in shape

In the first experiment, bees were trained using a blue square (positive) and a yellow square (negative), presented against a white background. This training situation encouraged the bees to learn to discriminate the colours, because the two shapes differed in no other parameter. However, during training, the bees had the option of learning, in addition, the shape of the rewarding figure.

Bees learned to discriminate between the blue and the yellow square not only very well but also very quickly. After one hour of training, CF for the blue square was 90.3% ($N=113$). The mean CF calculated from seven recording sessions conducted during the training in the course of the experiment was $89.0 \pm 4.3\%$ ($N=480$).

We subsequently exploited the trained bees' excellent colour discrimination performance to test them with further pairs of colours. In these tests, the possible influence of the learned shape was eliminated by using pairs of coloured triangles, rather than squares (Fig. 2). The bees discriminated blue very well from yellow and from green, but not from violet. Yellow and green were also discriminated very well from violet, but not from each other. The bees' choices between the various pairs of colours are very similar to those found in the wasp *Paravespula germanica* in another recent study (Lehrer and Campan, 2004), using the same pairs of colours. Although innumerable behavioural studies have focused on the colour

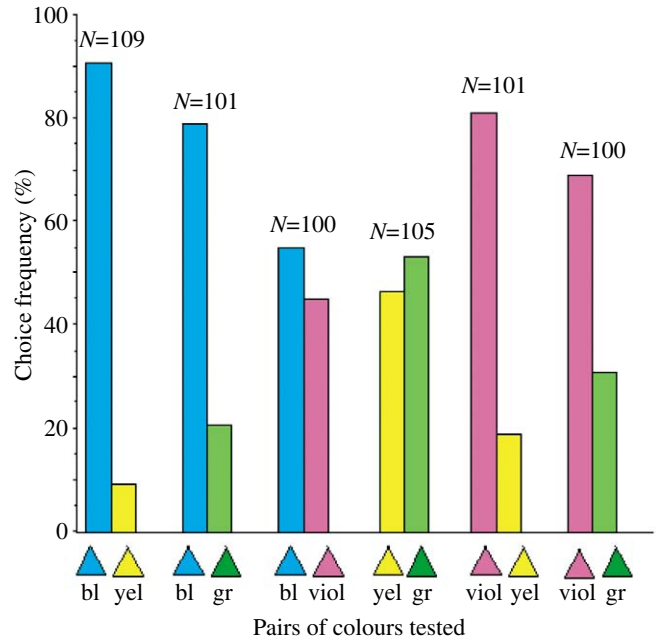


Fig. 2. Results of Expt 1, colour discrimination. Bees were trained with a blue square (positive) versus a yellow square (negative) presented on a white background. The trained bees are then tested with various pairs of coloured triangles as specified along the abscissa; bl, blue; yel, yellow; gr, green; viol, violet. The bars showing the choice frequencies (Cfs) obtained in favour of each of the two triangles are coloured accordingly. The total number of choices (N) recorded with each pair of triangles is given above each pair of bars.

discrimination performance of bees, and several on the colour vision of wasps (see references in Lehrer and Campan, 2004), the present experiments and those by Lehrer and Campan (2004) use, for the first time, exactly the same method, the same training shapes and the same coloured test stimuli for both of these hymenopteran species. In both species, colour discrimination is as expected from the spectral sensitivities of the three types of photoreceptors present in the retinas of these insects (Peitsch et al., 1992). The agreement between the choice behaviour of the two species documents the close correlation between the physiologically measured spectral sensitivities of the photoreceptors and the respective behaviourally measured colour discrimination performance.

However, colour was not the only parameter learned by the bees during the training. When the rewarding blue square was tested against each of four different novel blue shapes (a disc, a diamond and two different triangles), bees always preferred the square (Fig. 3, blue bars). In transfer tests presenting a yellow square against different yellow figures, bees again preferred the square (Fig. 3, yellow bars). The square was preferred even when all shapes were black (Fig. 3, black bars). Thus, bees have learned some spatial cue in addition to colour and use it in the discrimination task when they cannot use colour.

Summing up the results of this experiment, we find that (1) bees learn the shape of the rewarding figure in addition to its

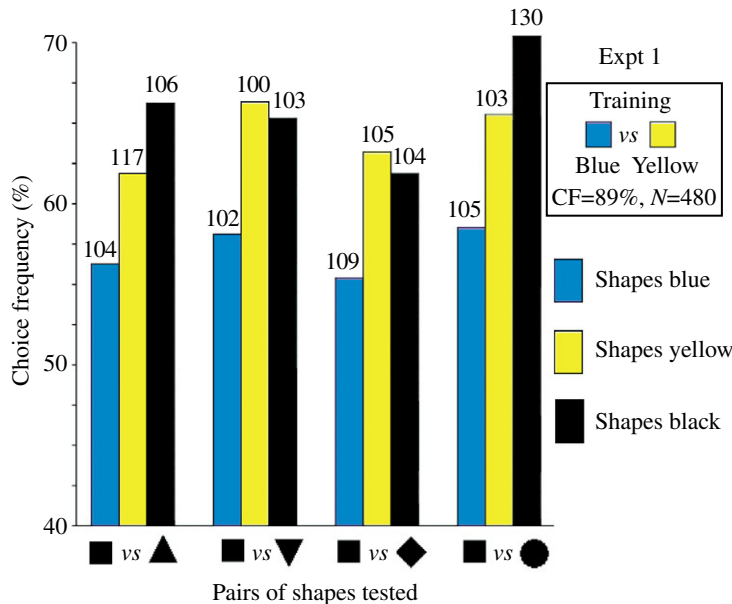


Fig. 3. Results of Expt 1, shape discrimination and generalization between colours. The same bees as in Fig. 2, trained with a blue square vs a yellow square. The inset shows the choice frequency (CF) obtained during the training and the number of choices (N) on which it is based. The trained bees were then offered a choice between a square and each of four novel shapes (abscissa). In the tests, both test shapes were either blue, as was the positive training shape, yellow, as was the negative training shape, or black, which was novel to the bees. The bars depicting the CF obtained in favour of the square are coloured according to the colour of the two shapes used in each test. The number above each bar denotes the total number of choices recorded in the test.

colour, even when training does not encourage shape learning, but that (2) colour is a more powerful cue than shape, at least in the present training conditions. The main result, however, is that (3) bees extract some feature contained in the rewarding stimulus and generalize this information to novel stimuli that produce a different colour contrast or an achromatic contrast against the background. This conclusion is supported by results of control tests showing that the positive training shape is preferred to the stimulus that was preferred in the transfer tests. In the tests with blue vs yellow, we already obtained (in the training) $CF=89.0\%$ (see above). A test of blue vs black (not illustrated) rendered $CF=80.2\%$ ($N=101$). Thus, the test shape was novel to the bees, showing that the choice behaviour is based on a generalization performance (see Introduction).

The relatively poor discrimination obtained with the blue shapes suggests that the colour of the learned figure was more important than any spatial cue. Evidently, the effectiveness of the parameter 'shape' was reduced due to the dominance of the parameter 'colour'. Indeed, when presented with a blue triangle against a black square, the bees preferred the blue triangle ($CF=77.4\%$, not illustrated) that had the wrong shape but the correct colour. We might conclude that the learned type of contrast plays a more important role than does the learned shape of the area, but we do not conclude so yet, because the training procedure employed in this experiment encouraged the

bees to learn a particular colour and not a particular shape. The additional role of the shape only became evident when the learned colour was absent in the tests, i.e. with the yellow and the black shapes. To examine the accuracy of shape discrimination, we must conduct a colour-neutral training experiment in which bees are encouraged to learn the shape, as will be the case in Expt 2.

Expt 2. Luminance contrast: training with a pair of shapes that differ in shape but not in colour

Whereas in Expt 1 bees were trained to discriminate between two figures that differed in colour but not in shape, now bees were trained using a pair of training figures that differ in shape but not in colour. The positive training shape was a black diamond, and the negative shape was a black disc. The aim of this experiment was to see whether or not this training procedure rendered a better shape discrimination performance than the previous training procedure and, by means of adequate transfer tests, whether or not the bees transfer the memory of the achromatic learned shape to shapes producing colour contrasts. In addition, because the shapes were placed at a distance in front of the background, it was also possible to examine the role of motion contrast and of covering parallax in the discrimination task. Finally, as in Expt 1, we wanted to place contrast and shape in competition with each other, to see whether the discrimination cue lies in the area or in the circumference of the shape.

The learning performance, calculated from six recording sessions, was convincing ($CF=69.4\pm 1.4\%$; $N=655$), although it did not reach such a high level as with colour learning ($CF=92.3\%$). This result agrees well with earlier results showing that shape learning in the bee is both slower and poorer than colour learning (see, for example, Lehrer et al., 1985).

Bees trained in this experiment discriminated between the training shapes not only in the training situation (Fig. 4A, black bar with white margin) but also when the two shapes were blue or yellow, rather than black, again presented against the white background (Fig. 4A, blue and yellow bar with white margins). Thus, the trained bees transferred the memory of the learned black shape producing achromatic contrast to coloured shapes producing colour contrasts.

The trained bees were subsequently tested with the diamond and the disc presented 5 cm in front of a black-and-white randomly patterned background. Four tests were conducted, with the pair of shapes being homogeneous black, homogeneous blue, homogeneous yellow or randomly patterned (four bars on the right-hand side in Fig. 4A). With the three pairs of homogeneous shapes, covering parallax was present in addition to luminance or colour contrast. With the patterned pair of shapes, only motion contrast was present.

The best result was obtained with the motion-contrast pair of shapes ($CF=82.2\%$, Fig. 4A, patterned bar with patterned

margins). Thus, bees transfer the information extracted from luminance contrast to stimuli that lack luminance contrast but offer motion contrast instead. The tests with the three pairs of shapes that produced covering parallax in addition to colour and luminance contrast (Fig. 4A, homogeneous shapes on patterned background) rendered a mean CF=70.6±3.6%. A very similar result was obtained with the same pairs of shapes on the white background (Fig. 4A, black, blue and yellow bars), although luminance contrast on the white background was higher than that on the patterned background. We conclude that adding covering parallax compensated for the

reduction in luminance contrast. Thus, covering parallax might play a role in the task.

The trained bees discriminated not only between the positive and the negative training shapes regardless of the type of contrast but also between the positive shape (the diamond) and a totally novel shape, the square, again regardless of whether black, blue or yellow figures were used (Fig. 4B).

In control tests (Fig. 4C), we find that bees discriminate the learned black diamond from a blue diamond, from a yellow diamond, from a black diamond on a patterned background and from a patterned diamond on the patterned background. Thus, the shape preferences obtained in the transfer tests constitute a true generalization performance.

We conclude from these results that (1) shape parameters are less effective than are colour cues, (2) covering parallax might compensate for reduction of luminance contrast and, mainly, (3) bees transfer shape information from achromatic to chromatic stimuli, as well as (4) to shapes that are perceived by pure motion contrast.

Finally, the trained bees were presented with a black disc *vs* a blue diamond, both on a white background. In this situation, the correct contrast was associated with the wrong shape, and the correct shape had the wrong contrast. Although the bees had been specifically trained to prefer the diamond and avoid the disc, in this test they preferred the disc to the diamond (CF=75.1%, $N=102$, not illustrated), showing that the type of contrast was learned and was, in addition, much more important than the shape of the area. We conclude that bees have generalized a cue that is located at the boundaries of the shape. We believe that the same conclusion is valid for the analogous test of Expt 1 (black square *vs* blue triangle).

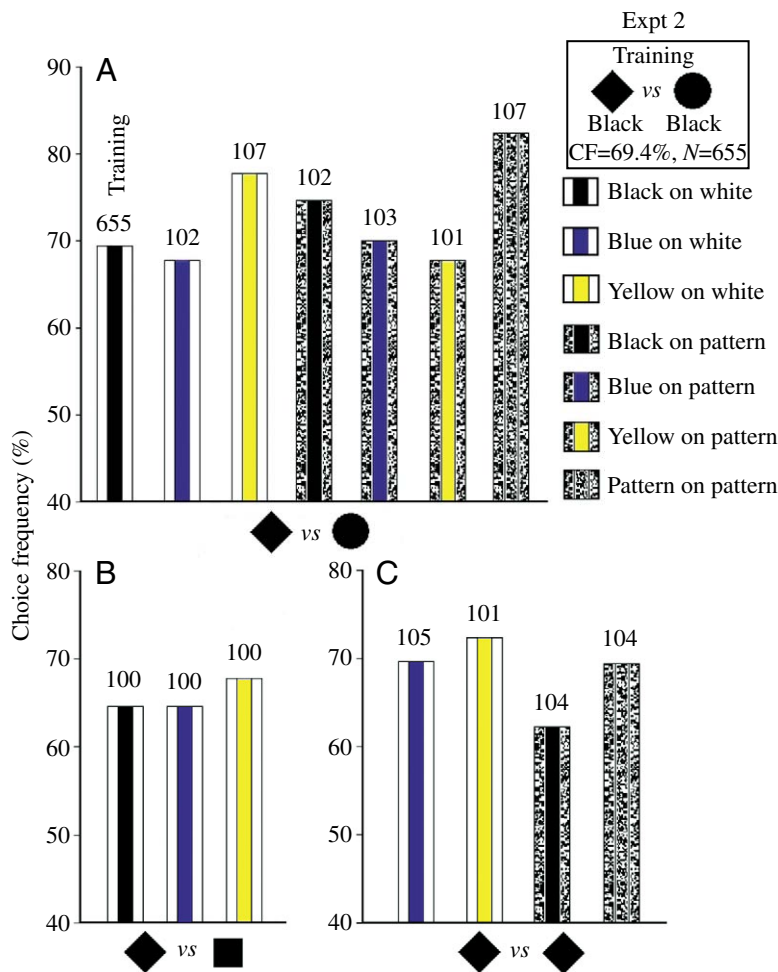


Fig. 4. Results of Expt 2, shape discrimination and generalization from luminance contrast to colour contrast and motion contrast. Bees were trained with a black diamond (positive) *versus* a black disc (negative) placed against a white background (inset). (A) The black-and-white bar on the left shows the choice frequency (CF) obtained during the training. Bees were then given transfer tests with a diamond *vs* a disc, which were both either blue or yellow, again on the white background (blue and yellow bar, respectively, with a white margin), and with black, blue and yellow shapes placed on a patterned background (bars with patterned margins). (B) Bees choosing between the learned diamond and a novel shape, a square, placed on a white background. The pair of test shapes is black, blue or yellow. (C) Control tests showing that the bees' choice behaviour constitutes a generalization performance, as explained in the text. In all three panels, the total number of choices (N) on which the CFs are based is given above each bar.

Expt 3. Training with a pair of shapes producing motion contrast against the background

In this experiment, the training shapes, this time a disc (positive) and a triangle (negative), carried a black-and-white random pattern, and so did the background. Due to the distance of 50 mm between the shapes and background, bees perceived relative motion between the figures and the background. Intensity contrast and colour contrast are absent in this constellation. The aim of the experiment was to examine whether generalization of shape occurs also in the opposite direction, i.e. from shapes learned using motion contrast to shapes that are recognized by using luminance or colour contrast. The role of covering parallax was examined as well.

As expected from the results of the transfer tests of Expt 2 (see Fig. 4A, patterned bars), bees learned the discrimination well. The mean result of five recording sessions was CF=70.6±3.5% ($N=533$), a result very similar to that obtained with the black training shapes

in Expt 2 (see Fig. 4A, black bar). Furthermore, the bees discriminated the learned patterned disc from various novel patterned shapes (Fig. 5A, abscissa), all presented against the patterned background. The results agree well with the results of shape discrimination tests obtained earlier (see Campan and Lehrer, 2002). Thus, bees seem to extract from motion cues very reliable information on the shape of the target.

The trained bees were now tested in six different transfer tests, using uniformly black, blue or yellow shapes presented either on a white background or on the patterned background (Fig. 5B). Two tests were performed in each of the six situations, presenting the disc either against the triangle (which was the negative shape during the training) or against a square, a shape that was novel to the bees.

In the tests with the white background, only colour or luminance contrast can be used to accomplish the discrimination, because no relative motion is perceived against a homogeneous background. In the tests with the patterned background, on the other hand, the bees could detect the homogeneous shapes not only by using luminance contrast or colour contrast but, in addition, by using covering motion.

In all these tests (Fig. 5B, black, blue and yellow bars), discrimination was highly significant, showing that bees transfer the shape information extracted using motion contrast to situations in which (instead of motion contrast) colour contrast and/or luminance contrast, as well as covering motion, is present. The mean CF obtained in the six transfer tests conducted with luminance contrast, and that obtained in the six transfer tests performed with covering motion (CF=65±2.8% and CF=67.1±3.2%, respectively) are practically the same. As in Expt 2 (see Fig. 4), the role of covering parallax is evident from the finding that the bees' performance does not deteriorate when the shapes are presented on the much darker patterned background.

As in the previous experiments, control tests were conducted (not illustrated) to make sure that we are observing a generalization performance. The positive training shape (the patterned disc on the patterned background) rendered CF=70.6% ($N=102$) against the uniform black disc, CF=68.6% ($N=102$) against the blue disc and CF=63.4% ($N=101$) against the yellow disc, all on the patterned background. Furthermore, when the bees trained to the motion contrast disc were given a choice between a patterned triangle and a homogeneous blue disc, both presented against the patterned background, they preferred the patterned triangle (CF=64.4%, $N=101$), although it had the shape of the negative training figure. Again, the type of contrast is more important than the shape.

Summing up, from the results of this experiment, we conclude that (1) a shape perceived on the basis of motion

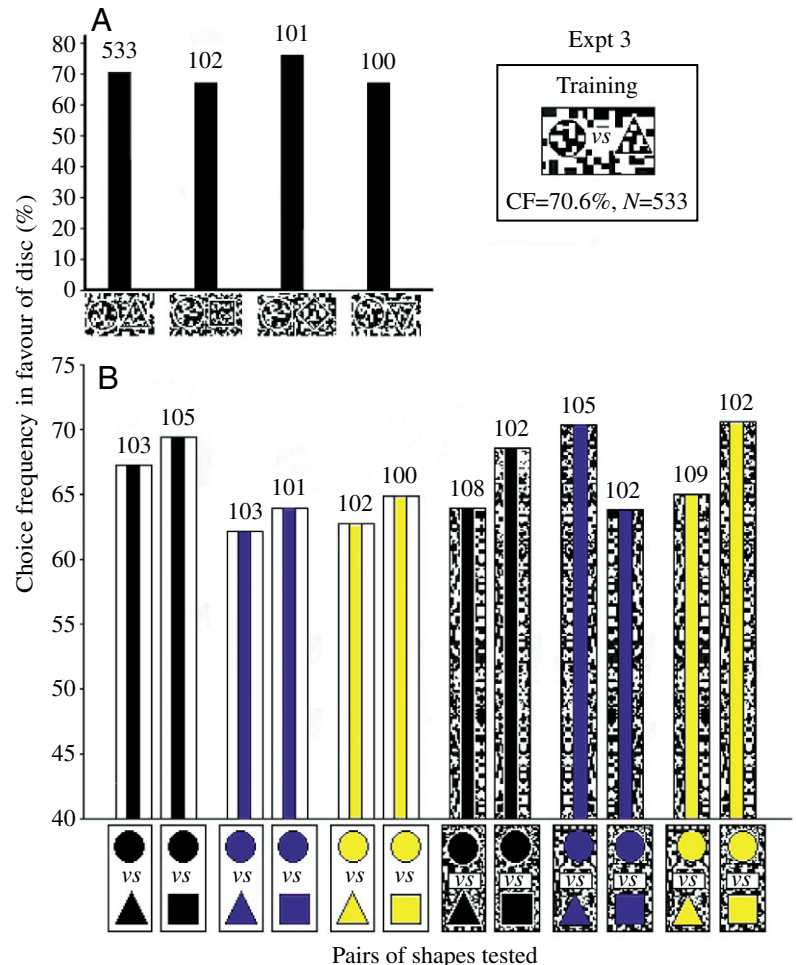


Fig. 5. Results of Expt 2, shape discrimination based on motion contrast, and generalization from motion contrast to luminance contrast and colour contrast. Bees were trained with a patterned disc (positive) versus a patterned triangle (negative) placed against a similarly patterned background. (A) Inset shows the choice frequency (CF) obtained during the training and the number of choices (N) on which it is based. The trained bees were then offered a choice between the trained patterned disc and four different novel patterned shapes, all placed on the patterned background. (B) The trained bees were given 12 different transfer tests, presenting black or coloured shapes either on a white background or on a patterned background. The bars denoting the CFs obtained in each test are coloured or patterned accordingly to the colour or pattern of the shapes used in the test, and the type of background is shown in the margins of each bar. In these tests, the disc was tested either versus a triangle, which was the negative training shape, or versus a square, which was novel to the bees (abscissa). The number above each bar denotes the total number of choices recorded in the test.

contrast is discriminated from the negative training shape as well as from totally novel shapes, all perceived on the basis of motion contrast, (2) generalization of pure motion contrast to covering motion is similar to the generalization from luminance contrast to covering motion (see Expt 2) and (3) bees generalize the learned shape from motion contrast to colour contrast and luminance contrast, i.e. shape generalization between luminance contrast and motion contrast occurs in both directions (see Expt 2).

Finally, in all three experiments, results of the special tests in which the area of the positive shape was tested against the type of contrast used in the training show that bees learn the type of contrast presented during the training but they do not associate the shape of the positive stimulus with the learned contrast.

The results thus support our original conjecture that the shape is not represented by its area but rather by some cue that is located at the boundary of the shape, which is the site where contrast occurs.

Expts 4 and 5: discrimination of contours

In the experiments that follow, we used two pairs of shapes, a disc and a triangle (Expt 4) and a square and a diamond (Expt 5). With each pair, two groups of bees were trained, one of the shapes being positive for one group and negative for the other. These experiments were conducted in Toulouse, using a slightly different set-up to that used in Expts 1–3 (see Materials and methods).

The shapes and the background were patterned during the training as well as during the tests. Thus, luminance contrast and colour contrast were absent, and bees had to rely on motion contrast to perceive the shapes. In the tests, the trained bees were presented with pairs of shapes that contained only the contours or fragments of the contours contained in the training shapes. The contours (or their fragments) were 2 cm wide, so that the random pattern they carried was intact. They were affixed to the background by means of 5 cm-long narrow sticks.

By using motion contrast rather than luminance or colour contrast, we allowed the bees to perceive the edges as independent shape elements. The areas that were missing in the test shapes gave a view of the background and therefore they moved to the bee's eyes with the same speed as the background, thus rendering them distinct from the edges, which moved faster. Bees that have learned to use edges in the discrimination task are expected to accomplish the discrimination even in these situations. With a homogeneous background, there is no cue by which bees could see that the test shapes possess no area.

The results of the reciprocal training experiments were very similar, i.e. the CFs obtained were fairly independent of whether one or the other shape of the pair served as positive. We therefore show only one experiment for each of the two pairs of shapes.

In either case (Fig. 6Ai,Bi), bees discriminated well between the two training stimuli when they were placed at the training distance (50 mm) but not when they were placed flat on the background (at zero distance), thus eliminating the contrast. Presenting just the circumference of the shapes or portions of it (Fig. 6Aii–v,Bii–viii) rendered, for all tested pairs, discrimination that was as accurate as that obtained in the training situation, in which the complete shapes were present

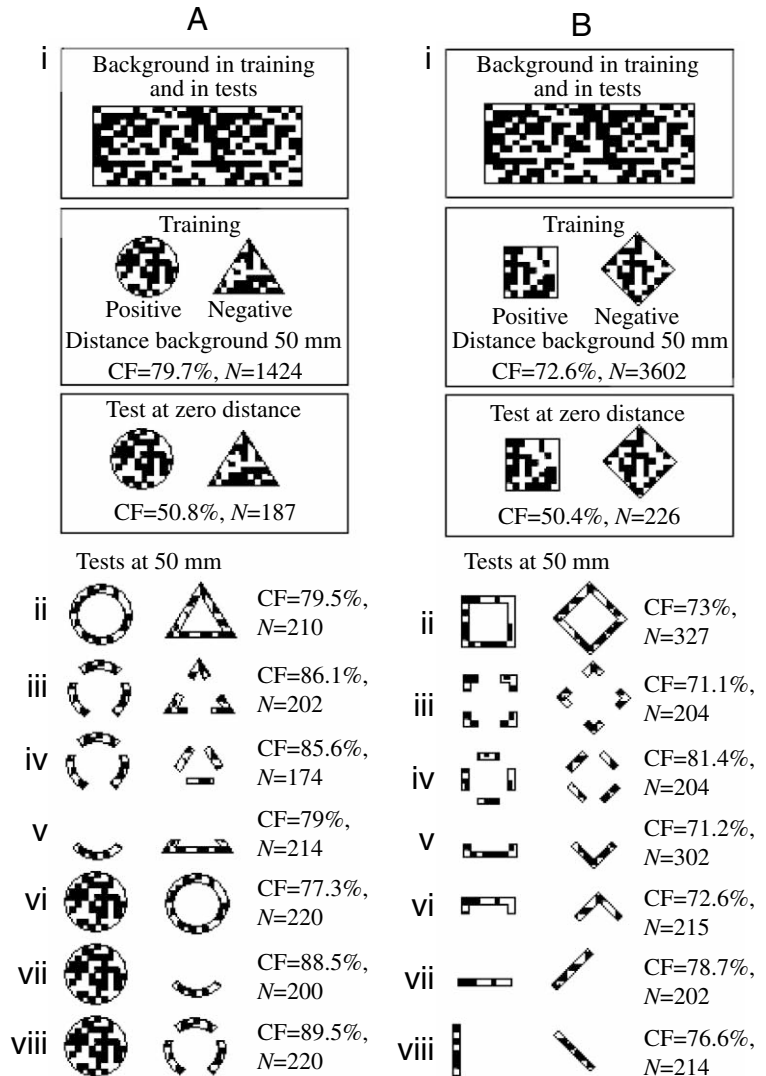


Fig. 6. Results of (A) Expt 4 and (B) Expt 5. Two pairs of randomly patterned convex shapes were used in two separate training experiments (A and B), presenting the shapes 50 mm in front of a patterned background (i, top). The shapes are shown separately for better visibility (i, middle). In this situation, bees could use only motion contrast for recognizing the shapes. When the distance between shapes and background is eliminated (i, bottom), bees cannot tell the two shapes apart. The trained bees are then tested using the outlines or fragments of the outlines of the two training shapes (ii–viii). Choice frequency (CF) and *N*-values are given for each test. Results of reciprocal training experiments (not illustrated) were very similar.

(Fig. 6Ai,Bi, distance 50 mm). Control tests (shown only for Expt 4) demonstrate that the bees discriminate the stimuli that were preferred in the transfer test from the positive test shape (Fig. 6Avi–viii), demonstrating that the bees' choice behaviour constitutes a generalization performance.

We conclude from the results that the cue used by the bees to discriminate among convex shapes is located at the circumference of the shapes. Contours, however, contain several features, all of which contribute to the appearance of a particular shape and are characteristic of it, such as the number, the length, the relative position and the orientation of edges,

presence or absence of corners and number and positions of corners. Any of these parameters might be extracted and used by the bee in the discrimination task. The present results do not identify any specific cue. Still, in almost all of the tests in Expts 4 and 5 (including those that are not illustrated here), discrimination between the disc and the triangle was better than that between the square and the diamond, which might be based on the fact that the two former shapes differ not only quantitatively but also qualitatively from each other: the disc does not contain corners and possesses no particular orientation of contours.

Do the bees just avoid the plugged tube?

In all the experiments, it was important to establish that bees could not differentiate between the two tubes. If they discriminated between the open and the plugged tube, then they would not need to rely on the shapes placed there for choosing the correct entrance.

The finding that CFs differed among different tests shows, rather convincingly, that the two tubes were not discriminated. This is because if bees could determine which of the tubes was plugged then they would avoid it and we would obtain a bias in favour of the open tube regardless of whether the positive shape was there or on the other tube. In this case, we expect CF to be close to 50% in all tests, because the positive shape was presented on the left and the right tube for equal durations of time. Sometimes, bees do develop a preference for one side, which is independent of the shape or of the tube. In almost all cases, however, the number of choices did not differ between the first and the second half of the test, showing that bees could not see (or did not mind) that one tube was blind.

We nevertheless tested the question directly by conducting, in Expts 1–3, control tests in which the shapes on the right and the left tube were identical. CF was calculated in favour of the open tube. In Expt 1, the blue square (which served as the positive training shape) was tested against itself (CF=51.0%; $N=104$) and in a test using two blue disks, which were novel to the bees (CF=51.0%; $N=104$). In Expt 2, a test with two black squares presented on a (novel) patterned background, CF was 52.9% ($N=102$). In Expt 3, CF was 55% ($N=100$) when the positive training shape was tested against itself.

Thus, bees can obviously not discriminate between the open and the blind tube. Indeed, the clear differences found among test results obtained using different shapes would not be expected if bees relied on cues other than those contained in the shapes. Therefore, the CFs obtained in the present study constitute a quantification of the bees' discrimination performance between the pairs of shapes tested.

Discussion

In recent years, many authors have used the occurrence of generalization performance to demonstrate the existence of cognitive capacities in the bee (see review by Menzel and Giurfa, 2001), comparable to such capacities in humans. In the present study, by examining generalisation of convex shapes,

our aim was not to demonstrate another cognitive behaviour in the bee. Instead, we were looking for direct evidence for the idea expressed in our previous paper (Campan and Lehrer, 2002), proposing that discrimination of convex shapes is accomplished by extracting a particular feature present in the rewarding shape, and that this feature does not involve the area of the shape. The generalization among different types of contrast only serves as another indication in favour of the idea that the cue used in the discrimination of convex shapes is located at the boundary of the shapes, a hypothesis supported by several further findings (see Introduction).

The present study asks whether or not bees trained to discriminate between two convex shapes producing a particular type of contrast transfer the learned information when they are presented with the same pair of shapes under novel contrast conditions.

The finding that bees generalize the shapes among different types of contrast shows that parameter extraction (see Introduction) is involved in the discrimination performance. We have already shown in a previous study (Campan and Lehrer, 2002) that bees do not use the alternative strategy, namely image matching, which involves the learning and recognition of the retinal positions of contrasting areas. In addition, the finding that the change in colour or pattern of the shape area does not affect the discrimination performance (Expts 1–3) suggests that the appearance of the shape area is not crucial, leaving only one alternative possibility, namely that bees memorize the outlines, rather than the areas, of the shapes. This conclusion is supported by the finding that discrimination does not deteriorate when the shapes are represented only by the outlines or portions of the outlines, the areas being absent altogether (Expts 4, 5).

Generalization among different types of contrast

The generalisation performance documented in the present study provides evidence that bees have learned a particular parameter present in the rewarding shape. The finding that the type of contrast does not matter in the present task is puzzling, because the various types of contrast used in the present study are governed by three distinct neural mechanisms. As described in the Introduction, colour perception results from comparing the excitations among the three spectral types of receptor, and luminance is monitored either by the output of one of the three spectral types of photoreceptor or by the summed excitations of all three. Motion, on the other hand, is perceived by a (usually directionally sensitive) movement detection system that receives its input from the green-sensitive (long-wavelength) receptors. It is not at all obvious how the bee's nervous system transfers visual information among the various types of contrast. One possibility would be that there exists a more central neural instance unto which all three paths converge and where they are somehow integrated to produce a common output. The other possibility would be that bees focus on the outline because they do not mind the appearance of the areas. Because the periphery of the shape is the site where contrast occurs, and because it is contrast that renders

the shape visible, the bees accept any type of contrast that they are able to perceive, even a novel one, just to make out the contours of the shapes.

The role of the training procedure

In all of the present experiments, we have employed a so-called differential procedure, meaning that during training bees were specifically encouraged to discriminate between two alternative stimuli, one associated with a reward, the other not. A variety of cues that are effective in discrimination tasks were found using this method (see Introduction). In our present experiments, however, the differential training only involved the shape of the training stimuli (Expts 2–5) and, in one case, their colour (Expt 1) and not the type of contrast, which did not differ between the positive and the negative shapes. Thus, bees were not specifically trained to learn the type of contrast. But they did.

This performance is not an exception. The honeybee's discrimination capacity is often independent of a differential training procedure, as demonstrated in several studies in which a single stimulus (rewarding) was presented during the training. For example, bees learned the colour (von Frisch, 1915; Daumer, 1956; Menzel, 1967) or the orientation of contours (e.g. Wehner, 1971, 1972a,b; Menzel and Lieke, 1983) despite the absence of a non-rewarding stimulus. Moreover, even when two stimuli are presented during training, learning does not always depend on the difference between them. Ronacher (1992) trained five groups of bees to a black disc (rewarding) using, in each training, one of five non-rewarding shapes that differed from the disc in either brightness, colour, 'dissectedness', size or outline (i.e. presence or absence of acute points). After training, each group of bees was subjected to dual-choice tests between the learned disc and several other shapes. The test results showed that size and outline are not learned unless a non-rewarding stimulus is presented during training that differs from the positive stimulus in size or outline, respectively. On the other hand, differences in colour, brightness and the degree of disruption were always effective in the discrimination task, regardless of whether or not bees have been differentially trained to use these parameters (Ronacher, 1992). In a more recent study by Lehrer (1999b), bees learned the orientation of the edge between two contrasting areas despite the fact that both training shapes displayed the same orientation of contours and only differed in colour. Although, in our present experiments, bees were not trained to discriminate between two different types of contrast, the rewarding contrast seems to have been learned because, in all tests in which contrast and shape are placed in competition, bees preferred the correct contrast to the correct shape. Nevertheless, they accept a novel type of contrast when the memorized contrast is absent in the test. Thus, bees learn the contrast in addition to shape without, however, having formed an association between the shape and the type of contrast under which it is viewed.

Colour contrast or green contrast?

The coloured shapes presented in the experiments certainly contain colour contrast because the background has a flat

reflectance curve (see Lehrer and Bischof, 1995) whereas the coloured papers used reflect different amounts of light at different wavelengths. However, they also contain receptor-specific contrasts, because the blue as well as the yellow papers we used reflect in both the short- and the long-wave portion of the spectrum (see table of values in Srinivasan and Lehrer, 1988). It has been shown several times that the green-sensitive receptors mediate motion detection (for reviews, see Lehrer, 1994, 1998). It is also known that even edge detection is based on the perception of green contrast (Lehrer et al., 1985, 1990; Zhang et al., 1995; Giger and Srinivasan, 1996) and that landing on edges is triggered by image motion (Lehrer and Srinivasan, 1993). Even the discrimination of convex shapes has been reported to require the presence of green contrast (Hempel de Ibarra and Giurfa, 2003). If this is indeed so, then the type of contrast that we thought was colour contrast is, in reality, green contrast. In this case, the different types of contrast among which bees generalize the shapes consist of luminance contrast, motion contrast and green contrast, instead of luminance contrast, motion contrast and colour contrast, but what we find still remains a generalization among different types of contrast.

We think, however, that our coloured stimuli were effective *via* colour contrast and not *via* green contrast. This is because all the studies examining the spectral properties of shape detection (e.g. Giurfa et al., 1996b, 1997; Hempel de Ibarra and Giurfa, 2003) have emphasized that green contrast is required only at large distances (i.e. when targets appear under small visual angles) whereas at close range, when the stimuli are larger (more than 15°), colour contrast is crucial. In two different studies involving discrimination of grating orientation at close range, bees accomplish the discrimination even in the complete absence of green contrast (Lehrer et al., 1985; Srinivasan and Lehrer, 1988). In a further study (Lehrer and Bischof, 1995), increasing the amount of green contrast did not improve the detection performance, and complete absence of luminance contrast did not matter for as long as colour contrast was present. Results reported by Niggebrücke and Hempel (2003) showed that the green system is very insensitive in the shape discrimination task. Horridge (2000), examining the role of green contrast in shape discrimination, suggested that green contrast is required when the bees have no fixation point whilst viewing the shape. In our experiments, bees had a fixation point (the opening of the tube) and, more importantly, at the decision point, i.e. upon reaching the entrance of the tube, they viewed the shapes at a very close range. By contrast, whenever bees were trained and tested in a Y-maze, they had to make decisions at a relatively far distance from the target. Under these conditions, green contrast was found to play a major role (see review by Giurfa and Lehrer, 2001).

The possible role of contour orientation

Although we have not identified the nature of the cue so far, we have reason to believe that it might be the spatial orientation of the contours. This parameter has repeatedly been shown to be very powerful in spatial discrimination tasks

(e.g. Van Hateren et al., 1990; Srinivasan, 1994; Srinivasan et al., 1994; Giger and Srinivasan, 1996; Horridge, 1997, 2000). In a differential training, bees are encouraged to learn to use edge orientation independently of the distribution of contrasting areas by randomizing the positions and sizes of the areas during the training, keeping the orientation constant. Bees trained in this way cannot use image matching in subsequent discrimination tasks and are forced to solely use contour orientation. However, bees learn contour orientation even without randomizing the areas (Wehner, 1971; Zhang et al., 1995), even in a non-differential training in which a negative stimulus is absent (Wehner, 1972a,b; Menzel and Lieke, 1983) or does not differ from the positive stimulus in the parameter under consideration (Lehrer, 1999b). Zhang et al. (1995) demonstrated that bees generalize the orientation cue between luminance contrast, green contrast and motion contrast, i.e. they transfer the information from one contrast condition to another, as in our present experiments, and we cannot exclude the possibility that the cue that the bees generalized was the same in both studies, namely contour orientation. The results by Zhang et al. render contour orientation a good candidate for serving the discrimination of convex shapes, which would agree with our conclusion that the critical cue is located at the edges. Indeed, convex shapes differ from one another in the orientation of their various contours more than in any other spatial cue. However, Zhang et al. (1995), being only interested in the cognitive nature of the behaviour, did not express the idea that the generalization of edge orientation might hint at the capacity of bees to use edge orientation in shape discrimination tasks.

How to answer the question?

Our present results do not reveal the nature of the cue and, so far, it could be any of several possibilities. It should be feasible, in the next step, to test cues such as position and number of edges and corners in isolation from one another. However, convex shapes possess one pair of cues that cannot be separated; the position of corners determines the spatial orientation of edges. If we find a generalisation of the orientation of edges, then this would indicate the role of the position of corners as well. Although it is possible to present edges of a particular orientation that are not associated with the presence of corners, i.e. single edges or gratings (see, for example, Wehner and Lindauer, 1967; Srinivasan, 1994; Zhang et al., 1995; Horridge, 2000), this will only tell us about the role of edge orientation, but little about its use in the discrimination of convex shapes.

Recently, Stach et al. (2004) have shown that a learned pattern containing gratings of three different orientations is discriminated from a pattern in which only one of these orientations has been altered, demonstrating the importance of edge orientation in shape discrimination tasks. In a convex shape, however, it is impossible to change the orientation of one edge without destroying the shape. To restore it, one would have to alter the orientation of other edges as well, or insert an additional edge.

One way of investigating the question would be in a series of experiments in which single edges contained in the positive shape are tested individually against single edges contained in the negative shape. Experiments of this type are in progress (M.L., manuscript in preparation).

The use of image motion

The results obtained in the present study show, as has already been shown with earlier results on the discrimination of convex shapes (Campan and Lehrer, 2002; Lehrer and Campan, 2004), that the insect uses self-induced image motion in the task of shape perception, at least when this is the only strategy that enables the detection of the shapes, i.e. when both the shapes and the background are patterned. Cues derived from image motion were shown to be used by the bee in a variety of visual tasks, such as separation of objects from their background, discrimination between moving and stationary objects, edge detection, and distance estimation (see references in Lehrer, 1994), all of which involve the use of depth information. The use of motion cues for 3-D orientation has also been found in solitary wasps at the nest entrance (Zeil, 1993; Zeil et al., 1996), in ground-nesting bees and wasps (Brünnert et al., 1994), in the locust (Wallace, 1959; Collett, 1978), in the larvae of the praying mantis (Walcher and Kral, 1994) and in a solitary bee (*Osmia rotundata*) orienting to a succession of landmarks leading to the nest (Fauria and Campan, 1998). In all of these behaviours, the stimuli contained luminance or colour contrast rather than motion contrast. In the bee, using coloured stimuli, the green sensitivity (and therefore colour blindness) of the behaviour was demonstrated whenever image motion served for coping with the task (see reviews by Lehrer, 1994, 1997). In our Expts 3–5, bees were forced to use motion contrast because other types of contrast were absent. However, there is little evidence that, when bees are not forced to use motion contrast, shape discrimination requires motion cues and is therefore colour blind. In the discrimination of grating orientation, Giger and Srinivasan (1966) report the exclusive use of green contrast, whereas Lehrer et al. (1985) and Srinivasan and Lehrer (1988) find little difference between results obtained with green contrast and those obtained with blue contrast. Hempel de Ibarra and Giurfa (2003), examining the discrimination of convex shapes in a Y-maze, report the exclusive use of green contrast, whereas Niggebrücke and Hempel (2003), again using a Y-maze, do not. Shape detection was found to be colour blind at small visual angles (Giurfa et al., 1996b, 1997; Giger and Srinivasan, 1996; Horridge, 1999) but not at angles larger than 15°. In none of the studies conducted in the Y-maze (Giger and Srinivasan, 1966; Giurfa et al., 1996b, 1997; Horridge, 1999; Hempel de Ibarra and Giurfa, 2003; Niggebrücke and Hempel, 2003) was it claimed that motion vision was involved, but it might of course have been. We cannot, so far, exclude the possibility that motion perception is not the only performance that is mediated by the green receptor. It might be involved in further spatial tasks that are not necessarily motion dependent, such as shape detection and discrimination.

Clearly, more experiments are needed. So far we can,

however, safely state that motion cues are useful even for shape discrimination when there is nothing but motion contrast that can be used. Apart from the honeybees, the use of motion contrast for shape discrimination has already been demonstrated, again using patterned constellations, in the leaf-cutter bee, *Megachile rotundata* (Campan and Lehrer, 2002), and most recently in a social wasp, *Paravespula germanica* (Lehrer and Campan, 2004). The particularly good performance of insects in using relative motion for shape discrimination might constitute an adaptation of the flying insect to its visual environment. In natural conditions, objects are hardly ever homogeneous, and the background is usually structured as well. The capacity to exploit motion parallax as well as covering parallax for detecting and recognizing objects against their background is therefore bound to be extremely useful to an insect moving within a natural scene.

We wish to thank two anonymous referees for careful reading and excellent comments.

References

- Anderson, A. M.** (1977). Parameters determining the attractiveness of stripe patterns in the honey bee. *Anim. Behav.* **25**, 80-87.
- Autrum, H.** (1957). Das Sehen der Insekten. *Studium Generale* **10**, 211-214.
- Backhaus, W. and Menzel, R.** (1987). Colour distance derived from a receptor model of colour vision in the honeybee. *Biol. Cybern.* **55**, 321-331.
- Baumgärtner, H.** (1928). Der Formensinn und die Sehschärfe der Bienen. *Z. vergl. Physiol.* **7**, 56-143.
- Brünnert, U., Kelber, A. and Zeil, J.** (1994). Ground-nesting bees determine the location of their nest relative to a landmark by other than angular size cues. *J. Comp. Physiol. A* **175**, 363-370.
- Campan, R. and Lehrer, M.** (2002). Discrimination of closed shapes in two bee species (*Megachile rotundata* and *Apis mellifera*). *J. Exp. Biol.* **205**, 559-572.
- Collett, T. S.** (1978). Peering – a locust behaviour pattern for obtaining motion parallax information. *J. Exp. Biol.* **76**, 237-241.
- Collett, T. S.** (1996). Honeybee navigation en route to the goal: multiple strategies for the use of landmarks. *J. Exp. Biol.* **199**, 227-235.
- Collett, T. S. and Cartwright, B. A.** (1983). Eidetic images in insects: their role in navigation. *Trends Neurosci.* **6**, 101-105.
- Collett, T. S. and Zeil, J.** (1997). The selection and use of landmarks in insects. In *Orientation and Communication in Arthropods* (ed. M. Lehrer), pp. 41-66. Basel, Boston, Berlin: Birkhäuser.
- Cruse, H.** (1972). Versuch einer quantitativen Beschreibung des Formensehens der Honigbiene. *Kybernetik* **11**, 185-200.
- Daumer, K.** (1956). Reizmetrische Untersuchung des Farbensehens der Biene. *Z. vergl. Physiol.* **38**, 413-478.
- Efler, D. and Ronacher, B.** (2000). Evidence against a retinotopic-template matching in honeybees' pattern recognition. *Vision Res.* **40**, 3391-3403.
- Ernst, R. and Heisenberg, M.** (1999). The memory template in *Drosophila* pattern vision in the flight simulator. *Vision Res.* **39**, 3920-3933.
- Fauria, K. and Campan, R.** (1998). Do solitary bees *Osmia cornuta* Latr. and *Osmia lignaria* Cresson use proximal visual cues to localize their nest? *J. Insect Behav.* **11**, 649-699.
- Frost, B. J., Wylie, D. R. and Wang, Y. C.** (1990). The processing of object and self-motion in the tectofugal and accessory optic pathways of birds. *Vision Res.* **30**, 1677-1688.
- Giger, A. D. and Srinivasan, M. V.** (1996). Pattern recognition in honeybees: chromatic properties of orientation analysis. *J. Comp. Physiol. A* **178**, 763-769.
- Giurfa, M. and Lehrer, M.** (2001). Honeybee spatial vision and floral display: From detection to close-up recognition. In *Cognitive Ecology of Pollination* (ed. L. Chittka and J. Thompson), pp. 61-82. Cambridge, UK: Cambridge University Press.
- Giurfa, M., Eichmann, B. and Menzel, R.** (1996a). Symmetry perception in an insect. *Nature* **382**, 458-461.
- Giurfa, M., Vorobyev, M., Kevan, P. and Menzel, R.** (1996b). Detection of colored stimuli by honeybees: minimum visual angles and receptor specific contrasts. *J. Comp. Physiol. A* **178**, 699-709.
- Giurfa, M., Vorobyev, M., Brandt, R., Posner, B. and Menzel, R.** (1997). Discrimination of colored stimuli by honeybees: alternative use of achromatic and chromatic signals. *J. Comp. Physiol. A* **180**, 235-244.
- Gould, J. L.** (1985). Pattern learning by honey bees. *Anim. Behav.* **34**, 990-997.
- Hempel de Ibarra, N. and Giurfa, M.** (2003). Discrimination of closed coloured shapes by honeybees require only contrast to the long wavelength receptor type. *Anim. Behav.* **66**, 903-910.
- Hertz, M.** (1929). Die Organisation des optischen Feldes bei der Biene. I. *Z. vergl. Physiol.* **8**, 693-748.
- Hertz, M.** (1930). Die Organisation des optischen Feldes bei der Biene. II. *Z. vergl. Physiol.* **11**, 107-145.
- Hertz, M.** (1933). Über figuralen Intensitäten und Qualitäten in der optischen Wahrnehmung der Biene. *Biol. Zbl.* **53**, 10-40.
- Horridge, G. A.** (1996). The honeybee (*Apis mellifera*) detects bilateral symmetry and discriminates its axis. *J. Insect Physiol.* **42**, 755-764.
- Horridge, G. A.** (1997). Pattern discrimination by the honeybee: disruption as a cue. *J. Comp. Physiol. A* **181**, 267-277.
- Horridge, G. A.** (1999). Pattern discrimination by the honeybee (*Apis mellifera*) is colour blind for radial/tangential cues. *J. Comp. Physiol. A* **184**, 413-422.
- Horridge, G. A.** (2000). Pattern vision of the honeybee (*Apis mellifera*): Blue and green receptors in the discrimination of translocation. *Neurobiol. Learn. Mem.* **14**, 1-16.
- Horridge, G. A.** (2003). Visual discrimination by the honeybee (*Apis mellifera*): the position of the common centre as the cue. *Physiol. Entomol.* **28**, 132-143.
- Horridge, G. A., Zhang, S. W. and Lehrer, M.** (1992). Bees can combine range and visual angle to estimate absolute size. *Phil. Trans. R. Soc. Lond. B* **337**, 49-57.
- Lehrer, M.** (1991). Bees which turn back and look. *Naturwissenschaften* **78**, 274-276.
- Lehrer, M.** (1993). Why do bees turn back and look? *J. Comp. Physiol.* **172**, 544-556.
- Lehrer, M.** (1994). Spatial vision in the honeybee: The use of different cues in different tasks. *Vision Res.* **34**, 2363-2385.
- Lehrer, M.** (1997). Honeybee's visual spatial orientation at the feeding place. In *Orientation and Communication in Arthropods* (ed. M. Lehrer), pp. 115-144. Basel, Boston, Berlin: Birkhäuser.
- Lehrer, M.** (1998). Looking all around: Honeybees' use of different cues in different eye regions. *J. Exp. Biol.* **201**, 3275-3292.
- Lehrer, M.** (1999a). Shape perception in the honey bee: Symmetry as a global parameter. *Int. J. Plant Sci.* **160**, 851-865.
- Lehrer, M.** (1999b). Dorsoroventral asymmetry of colour discrimination in bees. *J. Comp. Physiol. A* **184**, 195-206.
- Lehrer, M. and Bischof, S.** (1995). Detection of model flowers by honeybees: the role of chromatic and achromatic contrast. *Naturwissenschaften* **82**, 145-147.
- Lehrer, M. and Campan, R.** (2001). Discrimination of closed shapes in two bee species, *Apis mellifera* and *Megachile rotundata*. In *The Neurosciences at the Turn of the Century. Proceedings of the Göttingen Neurobiology Conference 28* (ed. N. Elsner and G. W. Kreuzberg), p. 693. Basel, Stuttgart, Berlin: Georg Thieme Verlag.
- Lehrer, M. and Campan, R.** (2004). Shape discrimination by wasps (*Paravespula germanica*) at the food source: generalization among various types of contrast. *J. Comp. Physiol. A* **190**, 651-663.
- Lehrer, M. and Srinivasan, M. V.** (1993). Object detection by honeybees: Why do they land on edges? *J. Comp. Physiol. A* **173**, 23-32.
- Lehrer, M., Wehner, R. and Srinivasan, M. V.** (1985). Visual scanning behaviour in honeybees. *J. Comp. Physiol.* **157**, 405-415.
- Lehrer, M., Srinivasan, M. V., Zhang, S. W. and Horridge, G. A.** (1988). Motion cues provide the bee's visual world with a third dimension. *Nature* **332**, 356-357.
- Lehrer, M., Srinivasan, M. V. and Zhang, S. W.** (1990). Visual edge detection in the honeybee and its spectral properties. *Proc. R. Soc. Lond. B* **238**, 321-330.
- Lehrer, M., Horridge, G. A., Zhang, S. W. and Gadagkar, R.** (1995). Shape vision in bees: innate preference for flower-like patterns. *Phil. Trans. R. Soc. Lond. B* **347**, 123-137.

- Manning, A.** (1957). Some evolutionary aspects of the flower constancy of bees. *Proc. Roy. Phys. Soc.* **25**, 67-71.
- Mazokhin-Porshnyakov, G. A.** (1969). Generalization of visual stimuli as an example of solution of abstract problems by bees. *Zool. J.* **48**, 1125-1135. (In Russian.)
- Menzel, R.** (1967). Untersuchungen zum Erlernen von Spektralfarben durch die Honigbiene (*Apis mellifica*). *Z. vergl. Physiol.* **56**, 22-62.
- Menzel, R.** (1987). Farbsehen blütenbesuchender Insekten. *Sonderdruck FU Berlin im Auftrag des Bundesministeriums für Forschung und Technologie.*
- Menzel, R. and Giurfa, M.** (2001). Cognitive architecture of a mini-brain: the honeybee. *Trends Cog. Sci.* **5**, 62-71.
- Menzel, R. and Lieke, E.** (1983). Antagonistic color effects in spatial vision of honeybees. *J. Comp. Physiol.* **151**, 441-448.
- Niggebrügge, C. and Hempel de Ibarra, N.** (2003). Colour-dependent target detection by bees. *J. Comp. Physiol. A* **189**, 915-918.
- Peitsch, D., Fietz, A., Hertel, H., de Souza, J., Ventura, D. F. and Menzel, R.** (1992). The spectral input systems of hymenopteran insects and their receptor-based color vision. *J. Comp. Physiol. A* **170**, 23-40.
- Ronacher, B.** (1979). Äquivalenz zwischen Größen- und Helligkeitsunterschieden im Rahmen der visuellen Wahrnehmung der Honigbiene. *Biol. Cybern.* **32**, 63-75.
- Ronacher, B.** (1992). Influence of unrewarded stimuli on the classification of visual patterns by honey bees. *Ethology* **92**, 205-216.
- Ronacher, B. and Duft, U.** (1996). An image-matching mechanism describes a generalization task in honeybees. *J. Comp. Physiol. A* **178**, 803-812.
- Srinivasan, M. V.** (1994). Pattern recognition in the honeybee: recent progress. *J. Insect Physiol.* **40**, 183-194.
- Srinivasan, M. V. and Lehrer, M.** (1988). Spatial acuity of honeybee vision and its chromatic properties. *J. Comp. Physiol. A* **162**, 159-172.
- Srinivasan, M. V., Lehrer, M. and Horridge, G. A.** (1990). Visual figure-ground discrimination in the honeybee: The role of motion parallax at boundaries. *Proc. R. Soc. Lond. B* **238**, 331-350.
- Srinivasan, M. V., Zhang, S. W. and Whitney, K.** (1994). Visual discrimination of pattern orientation by honeybees. *Phil. Trans. R. Soc. Lond. B* **343**, 199-210.
- Stach, S., Benard, J. and Giurfa, M.** (2004). Local-feature assembling in visual pattern recognition and generalization in honeybees. *Nature* **17**, 758-761.
- Van Hateren, J. H., Srinivasan, M. V. and Wait, P. B.** (1990). Pattern recognition in bees: orientation discrimination. *J. Comp. Physiol. A* **167**, 649-654.
- von Frisch, K.** (1915). Der Farbensinn und Formensinn der Bienen. *Zool. Jahrb. Allg. Zool.* **35**, 1-182.
- Walcher, F. and Kral, K.** (1994). Visual deprivation and distance estimation in the praying mantis larva. *Physiol. Entomol.* **19**, 230-240.
- Wallace, G. K.** (1959). Visual scanning in the desert locust *Schistocerca gregaria*. *Forskål. J. Exp. Biol.* **36**, 512-525.
- Wehner, R.** (1971). The generalization of directional visual stimuli in the honey bee, *Apis mellifera*. *J. Insect Physiol.* **17**, 1579-1591.
- Wehner, R.** (1972a). Pattern recognition in bees. *Nature* **215**, 1244-1248.
- Wehner, R.** (1972b). Dorsoventral asymmetry in the visual field of the bee *Apis mellifica*. *J. Comp. Physiol.* **77**, 256-277.
- Wehner, R.** (1981). Spatial vision in arthropods. In *Handbook of Sensory Physiology VII/6C* (ed. H. Autrum), pp. 287-616. Berlin, Heidelberg, New York: Springer.
- Wehner, R. and Flatt, I.** (1977). Visual fixation in freely flying bees. *Z. Naturforsch.* **32**, 469-471.
- Wehner, R. and Lindauer, M.** (1966). Die optische Orientierung der Honigbiene (*Apis mellifica*) nach der Winkelrichtung frontal gebotener Streifenmuster. *Zool. Anz. Suppl.* **30**, 239-246.
- Wolf, E. and Zerrahn-Wolf, G.** (1935). The effect of light intensity, area, and flicker frequency on the visual reactions of the honeybee. *J. Gen. Physiol.* **18**, 853-863.
- Zeil, J.** (1993). Orientation flights of solitary wasps (*Cerceris*; Sphecidae; Hymenoptera): II. Similarity between orientation and return flights and the use of motion parallax. *J. Comp. Physiol. A* **172**, 209-224.
- Zeil, J., Kelber, A. and Voss, R.** (1996). Structure and function of learning flights in ground-nesting bees and wasps. *J. Exp. Biol.* **199**, 245-252.
- Zerrahn, G.** (1934). Formdressur und Formunterscheidung bei der Honigbiene. *Z. vergl. Physiol.* **20**, 117-150.
- Zhang, S. W., Srinivasan, M. and Collett, T.** (1995). Convergent processing in honeybee vision: multiple channels for the recognition of shape. *Proc. Natl. Acad. Sci. USA* **92**, 3029-3031.