

## Chapter I

# How honeybees generalize visual patterns to their mirror image and left–right transformation

### Abstract

The ability to generalize a familiar image to visual transformations like a mirror image or a left–right transformation may allow recognition of familiar images from a different viewpoint. As this problem applies to flower recognition by honeybees, *Apis mellifera*, we asked whether bees transfer acquired information about a previously rewarded pattern to its mirror image and/or its left–right transformation, and which are the mechanisms involved in such a transfer. Bees were trained either with a single pair of patterns or with six different pairs of patterns presented in a random succession. Within each pair one pattern was rewarded and the other not. All patterns had four quadrants, each displaying a different stripe orientation. In multiple-pattern training the six rewarded patterns shared a common configuration different from that of the six nonrewarded ones. After both kinds of training, the bees preferred the mirror image and the left–right transformation of the rewarded pattern (or rewarded configuration) to a novel pattern. They also preferred the left–right transformation to the mirror image. We explain this performance by: (1) matching with a retinotopic template of the trained patterns after training with a single pair of patterns; and (2) matching with a generalized pattern configuration after training with a randomized series of patterns. In the second case, orientations would be bound together in a specific spatial arrangement. Bees would associate a specific orientation with each retinal quadrant and approach the pattern provided that a particular quadrant contains a particular orientation. Although both strategies are based on comparison of an image currently perceived with one that has to be accessed from memory, they constitute different options as the former is less flexible while the latter allows for categorization of novel patterns.

## Introduction

Bees are flower constant, that is, they return to flowers of the same species provided that these are profitable (Menzel, 1985; Waser, 1986; Chittka et al., 1999). To this end, bees learn and memorize not only the location of the food sources but also the specific floral features that allow them to discriminate between rewarding and nonrewarding flowers. Visual cues are of fundamental importance in this context (von Frisch, 1967; Dafni et al., 1997a, b; Giurfa & Menzel, 1997; Giurfa & Lehrer, 2001). Among them, shapes and patterns play an important role in close-up recognition (Wehner, 1981; Srinivasan, 1994; Giurfa & Lehrer, 2001). Owing to the relatively poor resolution of the insect compound eye, such visual cues drive the choice behaviour of flying bees only close to the flowers (Wehner, 1981; Vorobyev et al., 1997). Nevertheless, the great variety of existing floral shapes and patterns suggests that even at this range, shape discrimination is an important component of the bee's decision process. This assumption is confirmed by many behavioural experiments showing pattern discrimination in bees at this stage (Wehner, 1981; Giurfa & Lehrer, 2001).

In a natural environment, a corolla may be partially covered or occluded by leaves or other objects such that a bee has to identify the learned global image from the actual incomplete image (Gould, 1988). The bee faces a similar problem if it approaches the flower from a different direction. In all these cases the actual flower view is different from the learned view. Changes can involve the mirror image or other kinds of visual transformations (e.g. rotations) of the original view. For instance, if bees forage on a bilateral symmetrical flower whose right half is hidden by leaves, they should be able to recognize the same flower if its left part is now hidden or if the whole corolla is visible. The ability to generalize a familiar image to such visual transformations is necessary to solve the uncertainty implied in these problems. Such an ability has been shown in some

vertebrate species (pigeons, *Columba livia*: Hollard & Delius, 1982; goldfish, *Carassius auratus*, and rats, *Rattus* sp.: Sutherland, 1969; cats, *Felis catus*: Warren, 1969; sea lions, *Zalophus californianus*: Mauck & Dehnhardt, 1997). Generally, it is assumed that the underlying mechanisms allowing such generalizations have an important cognitive basis. For instance, in generalizing to a rotated image, mental rotations of the learned image are assumed to occur in the brain (Shepard & Metzler, 1971; Cooper & Shepard, 1973; Cooper, 1975; Cooper & Podgorny, 1976; Hollard & Delius, 1982).

The generalization of a learned image to its different visual transformations can be studied using the honeybee as a model system. Bees learn and memorize shapes and patterns in a very efficient way and can therefore be tested for discrimination between a trained stimulus and a given transformation. Learning of visual cues can surpass elementary associations and can be consistent with cognitive phenomena such as categorization, the building of identity relationships, occasion setting and configural forms of learning (for review see Giurfa & Menzel, 1997; Menzel & Giurfa, 2001). Therefore, the question of whether bees can generalize acquired information about a trained pattern to its mirror image or to its left–right transformation (transposition of the left and right sides of the pattern) is pertinent.

Such a question was first raised by Gould (1988) using honeybees. Later, Korneluk & Plowright (1995) and Plowright (1997) studied the same problem using bumblebees *Bombus impatiens*. Both studies claimed that bees have a facultative ambiguity for mirror-image transformations, that is, they distinguish the mirror image and the original stimulus in a discriminative task but prefer the mirror image to a novel stimulus in another discriminative test. However, these results are doubtful because of questionable experimental design. In Gould's (1988) study, each stimulus consisted of four circles, each 2.5 cm in diameter and a different colour (white, yellow, orange and blue for humans). The

transformations were obtained by changing the positions of the circles. The use of different colours in this context is questionable because bees have preferences for certain dominant wavelengths (Giurfa et al., 1996a), in particular, blue and yellow (around 410 nm and 530 nm, respectively) and also learn such wavelengths faster (Menzel, 1967). Furthermore, bees can confuse the similar yellow and orange. Gould did not control for these effects and may have introduced sensory biases towards specific parts of the stimuli in which a salient colour was presented. When confronted with novel permutations such as a mirror image, the bees would always prefer the permutation displaying the best match with the previously learned arrangement of colours. No mirror image ambiguity is required to explain Gould's results.

In Korneluk & Plowright's (1995) experiments, the colours were slightly different (white, blue, green and yellow) but, again, the possibility of learning the stimuli in terms of the position of preferred colours was not excluded. Plowright (1997) used different stimuli which, from the human perspective, would allow one to distinguish between a trained pattern, its mirror image and its left–right transformation but their design was inappropriate. They consisted of four yellow circles (2.5 cm in diameter) each having a different distinctive shape drawn in its centre (<, d, c or ■; see his Figure 1). Appropriate permutations of the shapes result in distinct alternatives for the left–right transformation and the mirror image of the trained pattern. However, these shapes were very small (ca. 2 mm after Plowright's Figure 1) and theoretical calculations (see Giurfa et al., 1995; 1997; Vorobyev et al., 1997) show that, even at a short distance (4 cm from the targets), bees could not resolve the shapes and could not therefore discriminate between a trained stimulus and any kind of transformation. Nonvisual cues such as the position of the experimenter may have allowed the bees to solve the discriminations.

In our experiments we used stimuli whose transformations resulted in different

alternatives for mirror image and left–right permutation. To exclude possible perceptual asymmetries between colours, we used achromatic (black-and-white) patterns. Furthermore, we took into account the resolution capabilities of the bee’s eye, such that all pattern elements could be perfectly resolved by the bees at the distance at which they had to decide between patterns (15 cm). Finally, to ensure that bees were learning the trained patterns as a whole and not only parts of them, as can sometimes happen (see Giurfa et al., 1999 for review), we always presented each rewarding pattern with a nonrewarding one in a differential conditioning procedure. Such a training procedure guarantees that bees learn the rewarded pattern as a whole (Giurfa et al., 1999).

We investigated whether bees can transfer the information acquired about a previously rewarded pattern to its mirror image and/or its left–right permutation. In answering this question we analysed the mechanisms involved in such transfer strategies.

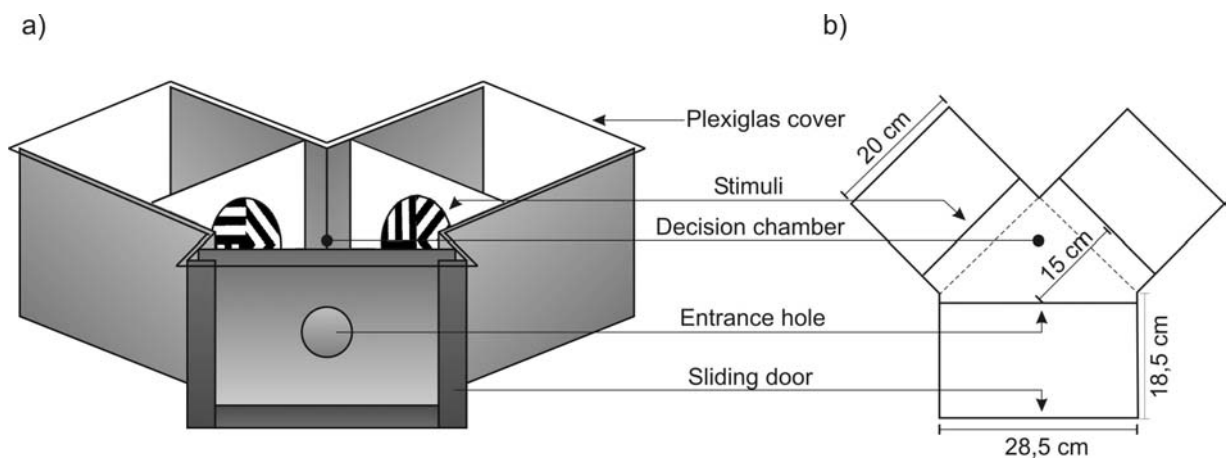
## **Methods**

### **Apparatus**

Individually marked honeybees, *Apis mellifera carnica*, were trained to collect 50% sucrose solution (weight/ weight) on vertically presented stimuli lying flat on the back walls of a wooden Y maze (Fig. 1; for more detailed information about the apparatus and the procedure see Giurfa et al., 1999). The apparatus was covered with a UV-transparent Plexiglas ceiling to ensure natural= daylight illumination within the maze.

The Y maze was placed close to a large open window in the laboratory, through which bees could enter from the outside and to ensure natural illumination of the set-up. The bee entered the maze through an entrance hole (5 cm in diameter) in the front panel. This hole could be opened or closed with a sliding door to guarantee that only the bee under study was present in the maze at any one time. Once the bee passed the entrance hole, it reached

the decision chamber from which it could see for the first time both back walls of the maze simultaneously. Each back wall presented a circular black-and-white pattern on a white background. Only one of them (termed positive) presented a reward of sucrose solution whilst the other (termed negative) did not. The side of the positive and negative stimuli was interchanged following a pseudorandom sequence (see Giurfa et al., 1999) to avoid the bee learning positional information.



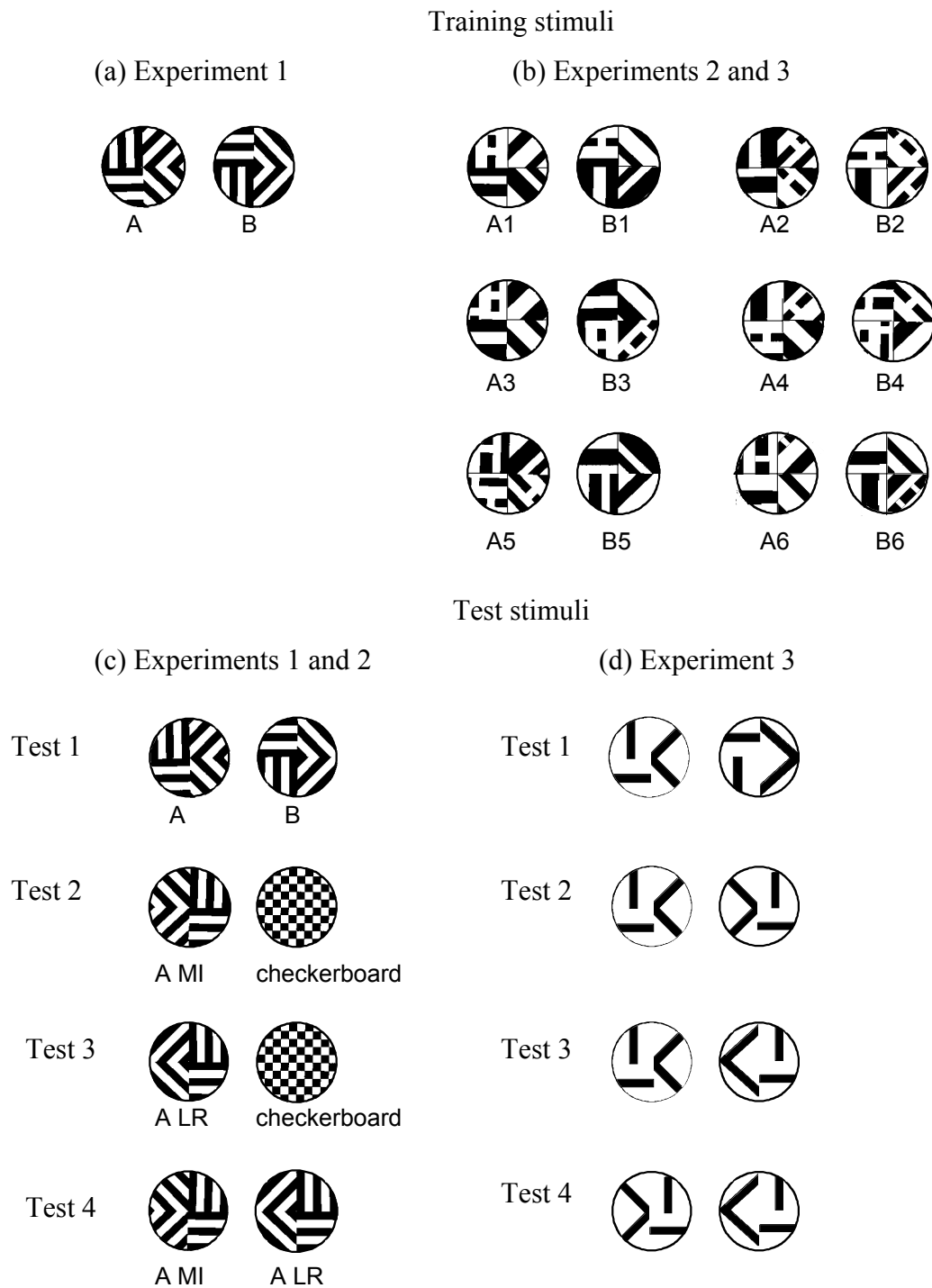
**Figure 1.** a) Front view of the Y maze apparatus. Bees entered the maze through a sliding door and faced a wall with a central hole, 5 cm in diameter, through which they gained access to the decision chamber. There they faced two black-and-white patterns presented vertically on the back walls of the arms of the maze. One of the patterns was rewarded with sucrose solution and the other was nonrewarded. The whole apparatus was covered with an ultraviolet-transmitting Plexiglas ceiling. b) Overall view and dimensions of the set-up.

### Stimuli

The stimuli (Fig. 2) were black-and-white circular patterns, 10 cm in diameter. They were produced with a high-quality laser printer on white copying paper of constant quality. The

patterns were placed 15 cm from the entrance hole such that they subtended a visual angle of  $37^\circ$  to the eye of a bee entering the decision chamber. The visual angle at the decision point (imaginary point in the middle of the decision chamber) was  $44^\circ$  (for details on calculation of the visual angle see Giurfa et al., 1995).

Each training pattern was divided into four quadrants each of which presented black-and-white gratings with a particular orientation ( $0^\circ$ ,  $45^\circ$ ,  $90^\circ$  or  $135^\circ$  with respect to the vertical). The orientations chosen can be discriminated from each other by honeybees (Srinivasan et al., 1994). The width of the stripes varied depending on the experiment but in all cases it was at least 1 cm. Such a minimal width corresponds to a visual angle of  $4^\circ$  for a bee entering the maze and was resolvable for the bee's eyes (Srinivasan & Lehrer, 1988). The use of four-quadrant patterns with four different orientations allows one to permute the quadrants to generate different alternatives for a mirror image and a left-right transformation (see example in Fig. 3).



**Figure 2.** Stimuli used in the experiments. (a) Training stimuli used in experiment 1. (b) Training stimuli used in experiments 2 and 3. In both experiments, the different pairs of patterns were presented in a randomized succession. (c) Test stimuli and tests in experiments 1 and 2. Tests after training on the A-type patterns are shown as an example. (d) Test stimuli and tests in experiment 3. Tests after training on the A-type patterns are shown as an example.





**Figure 3.** Visual transformations of a trained pattern as illustrated by pattern A. The use of four different orientations allowed us to permute the quadrants to generate different alternatives for a mirror image (MI) and a left–right (LR) transformation.

### Procedure

Depending on the experiment, bees were trained either with only one pair of patterns (experiment 1; Fig. 2a) or with a randomized succession of six different pairs of patterns (experiments 2 and 3, Fig. 2b). The conditioning procedure was always differential. If the bee chose the correct pattern, it was rewarded with sucrose solution dispensed by a microsyringe in the middle of the pattern. If the bee chose the wrong pattern, we tossed it away from the maze by hand and it had to re-enter through the entrance hole and make a new decision. This was repeated until the bee chose the correct pattern but we counted only the first choice. In that way, wrong decisions were penalized and choice performance improved.

In each experiment, we separately trained and tested two groups of bees. Bees used in one experiment were killed in 90% alcohol to avoid reusing. The stimulus type that was rewarding for one group was nonrewarding for the other group and vice versa. By performing reciprocal training and tests, we could detect preferences for one stimulus type, if any.

We stopped the training after 42 learning trials (i.e. 42 presentations of the training stimuli or 42 visits of the bee to the maze). This amount of training was sufficient for bees to learn a similar task with similar stimuli (Giurfa et al., 1999) and was also sufficient in

this set of experiments. For experiments 2 and 3, in which six pairs of training stimuli were used, each pair was presented seven times in a random sequence.

Once the bees completed the training, they were tested in extinction conditions (i.e. no reward was provided on the test stimuli). In such tests, we presented fresh patterns on exchangeable walls for 2 min to the bee under study. Each test was done twice, with the sides of the stimuli exchanged from one test to the other. In this way, we were able to control for position tendencies in the choice of patterns.

During each test we counted the number of touches for each stimulus (flights towards a pattern that ended with the bee touching it with its antennae). Relative choice frequencies for each test pattern were calculated from the absolute number of touches registered. Approaches (flights towards the pattern that did not end with the bee touching it) were not taken into account as they constitute an insensitive parameter to uncover the choice strategies of bees in these circumstances (Giurfa et al., 1999). In all cases, training and test patterns were regularly replaced by fresh ones to avoid influences of olfactory cues.

## **Experiment 1**

We used a single pair of patterns (A and B, Fig. 2a) to train the bees. Stripe width was always 1 cm. The training patterns differed markedly from each other because the stripe orientation of each of the four quadrants was perpendicular from one pattern to the other, thus reducing to a minimum the overlap between them. Figure 2c shows the corresponding test patterns. We did four tests to determine whether bees prefer the mirror image (MI) or the left–right transformation (LR) of a trained pattern to a novel stimulus (a chequerboard, Ch) in the absence of the trained pattern. The chequerboard had squares of 1 x 1 cm. Each square thus had the same width as the grating stripes. The chequerboard is a

useful stimulus in this kind of experiment because bees do not necessarily prefer a pattern with stripes to a chequerboard (Giurfa et al., 1999). For instance, if the chequerboard is presented with a nonrewarding striped pattern, similar to the ones we used, bees prefer the chequerboard. In the first test we presented the bees with the training stimuli to verify whether they had learned the training task. In the second test, bees were presented with the mirror image of the rewarded pattern versus the chequerboard. In the third test, the left–right transformation of the rewarded pattern was presented with the chequerboard. Finally, in the fourth test, the mirror image and the left–right transformation were presented.

## **Experiment 2**

In this experiment we excluded the possibility of the bee learning the training patterns in terms of a retinotopically fixed template (Wehner, 1972; Gould, 1985; 1986). In the previous experiment the bees could memorize the single pair of patterns and choose between the test alternatives by the best match with the template. This low-level mechanism has to be ruled out to study more flexible generalization performances. Thus, in experiment 2, we trained bees with six pairs of different training patterns (Fig. 2b) and randomized the presentation sequence during the 42 learning trials. In this way, template building was prevented (Giurfa et al., 1996b). After training, we tested bees as in experiment 1 (Fig. 2c). In this case, all test stimuli were new to the bees.

The training patterns were also divided into four quadrants with different orientations. The width of the stripes varied from one quadrant to the other and from one pattern to the other. However, in all cases, the stripes were resolvable for the bees (1, 1.5 and 2 cm width). Despite their variation in stripe width, the rewarding, as well as the nonrewarding, patterns shared a specific layout with respect to their available orientations. Two common layouts could be characterized: one for the rewarding stimuli and another for the nonrewarding

ones (e.g. all stimuli A in Fig. 2b presented a 0° orientation in the upper left quadrant, a 90° orientation in the lower left quadrant, a 45° in the upper right quadrant and a 135° in the lower right quadrant). Stacking the six stimuli sharing a common layout resulted in a solid black circle. This is because stacking two black stripes on each other and stacking a black stripe on a white one both result in a black stripe, as the overall luminance is the same in both cases. Luminance is defined as the sum of the excitations of all three photoreceptor types, S, M and L. Luminance differences are not relevant for bees as they do not possess a perceptual dimension for luminance (or intensity; Menzel & Backhaus, 1991). Bees cannot distinguish two yellows with different luminances if they are close to the target (at the range at which patterns are perceived; Giurfa et al., 1997). Pattern perception by bees could be mediated, not by a luminance channel, but by the S-receptor channel alone (Giurfa et al., 1997).

### **Experiment 3**

The training procedure was the same as in experiment 2 (Fig. 2b). However, the orientation information of the test stimuli was reduced to a single bar per quadrant (Fig. 2d). Each bar was 1 cm wide. In the first test, one pattern had the same layout as the rewarding stimuli, whilst the other had the layout of the nonrewarding stimuli. In the second test, the patterns were the layout of the rewarding stimuli and its mirror image. In the third test, the patterns were the layout of the rewarding stimuli and its left–right transformation. Finally, in the fourth test, the mirror image and the left–right transformation were presented.

### **Statistics**

We used one-way ANOVA to determine whether the two groups of bees reciprocally

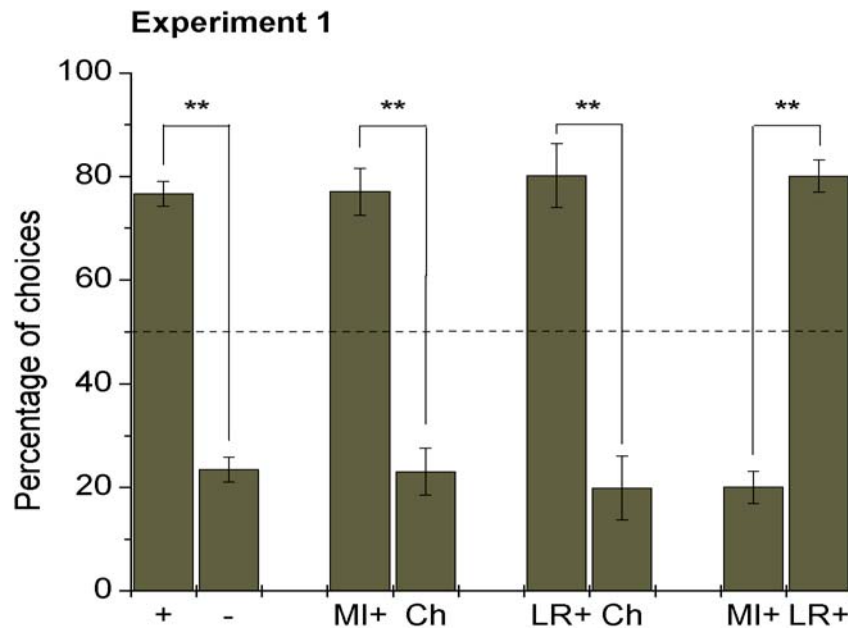
trained and tested in each experiment (see procedure) differed in their performance. If not, their data were pooled. Preference for one pattern within a pair of test stimuli was analysed with a two-tailed binomial test using a single choice value for each bee.

## Results and Discussion

### Experiment 1

#### *Results*

We asked whether bees prefer the mirror image or the left–right transformation of a trained pattern to a novel stimulus (a chequerboard) in the absence of the trained pattern. We trained 15 bees with a single pair of patterns A and B (Fig. 2a), with A being either the positive (nine bees) or the negative pattern (six bees; B then being the negative or the positive pattern, respectively). Since no significant difference was found in the performance of the two groups of bees trained and tested in this experiment (A+ B\_ versus A\_ B+; one-way-ANOVA:  $F_{1,13} = 0.10$ , NS), we pooled the data of the two groups. In the first test (Fig. 4) the bees were presented with fresh patterns identical to those used in the training. The bees significantly chose the pattern previously rewarded, thus showing that they had learned the training task (+ versus -: two-tailed binomial test:  $N=15$ ,  $P < 0.00005$ ). In the second test, bees significantly preferred the mirror image of the rewarded pattern to the chequerboard (MI+ versus Ch:  $N=15$ ,  $P < 0.001$ ). In the third test, bees preferred the left–right transformation of the rewarded pattern to the chequerboard (LR+ versus Ch:  $N=15$ ,  $P < 0.001$ ). Thus, bees perceived both pattern transformations, mirror image and left right, as being more similar to the trained pattern than the chequerboard. In the fourth test the transformations were not equally ranked by the bees. When the mirror image and the left–right transformation were presented together, bees significantly preferred the latter (MI+ versus LR+:  $N=15$ ,  $P < 0.00005$ ).

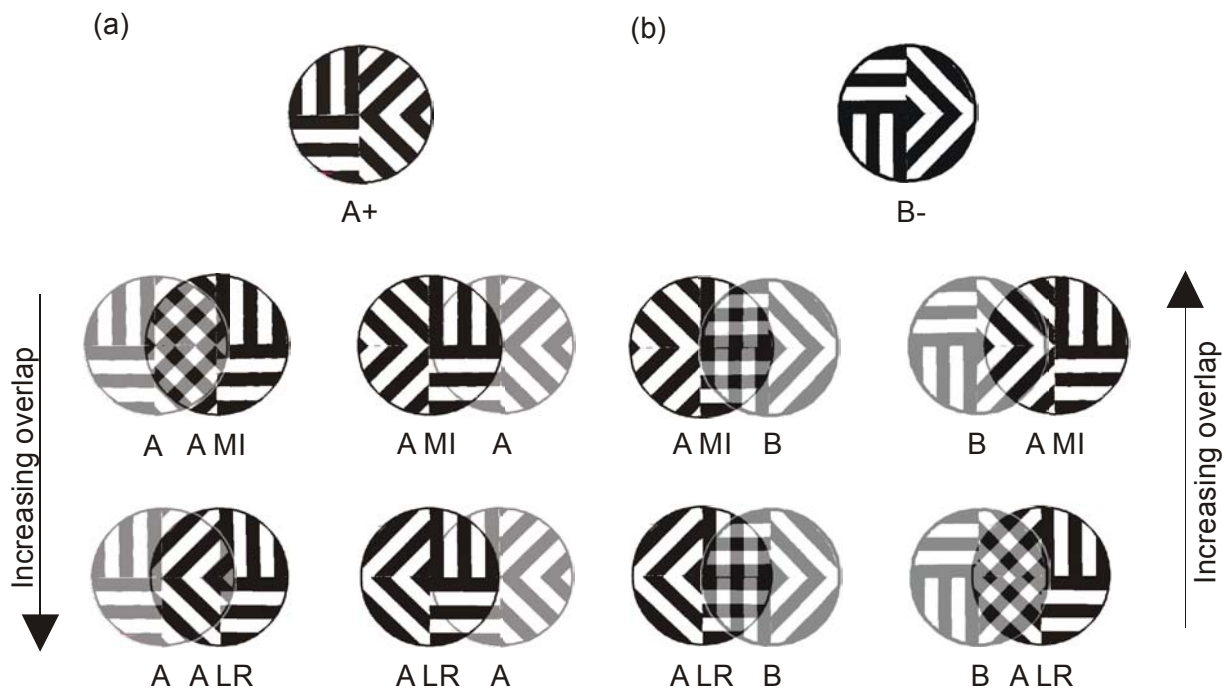


**Figure 4.** Percentage of choices in the different tests of experiment 1. Under each column the test alternative is shown: MI=mirror image; Ch=chequerboard; LR=left–right transformation. Values are  $\bar{X} \pm SE$  from 15 bees.  $**P < 0.001$ .

### Discussion

These results show that bees can generalize a trained pattern to its mirror image as well as to its left–right transformation when the trained pattern is absent. However, before we conclude that bees possess a mirror-image ambiguity or a left–right transformation ambiguity we need to consider more parsimonious explanations based on low-level mechanisms of visual recognition. Figure 5 shows that the bees could have learned the positive pattern as a template (in Fig. 5, pattern A, in grey). A bee could match the template of the trained positive pattern with both the left and the right halves of the left–right transformation but with only one half of the mirror image (Fig. 5a). We know that in differential training procedures both the rewarding and the nonrewarding patterns are learned (Giurfa et al., 1999). Therefore a template of the nonrewarding pattern can also be

used for deciding between patterns: the more the coincidence between a perceived pattern and a negative template, the less the choice of the perceived pattern (Fig. 5b). As the mirror image contains half the rewarding and half the nonrewarding stimulus (see Fig. 5), and the bees prefer it to a neutral chequerboard (test 2), this implies that the signal from matching positively filled quadrants is stronger than the avoidance signal from matching negatively filled quadrants.



**Figure 5.** Template-based recognition as an explanation of the results of experiment 1. In this case, pattern A was taken as the rewarded pattern and it is assumed that bees learned that pattern as a retinotopically fixed template. The transparent pattern represents the positive (a) and the negative (b) pixel-based templates available in memory. The black-and-white patterns are the pattern transformations actually perceived. (a) The overlap between the positive template and its left–right (LR) transformation is higher than that between the positive template and its mirror image (MI). (b) The overlap between the negative template and the left–right transformation of the rewarded pattern is lower than that between the negative template and the mirror image of the rewarded pattern.

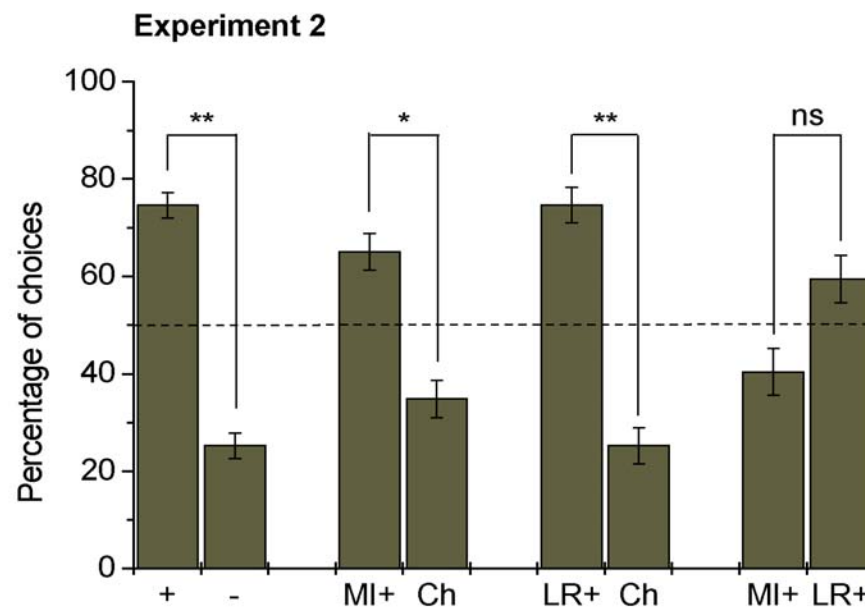
## Experiment 2

### *Results*

No significant differences were found in the performance of the two groups of bees reciprocally trained in this experiment (one group of seven bees rewarded on stimuli A1–A6 and nonrewarded on stimuli B1–B6, and another group of nine bees rewarded on stimuli B1–B6 and nonrewarded on stimuli A1–A6:  $F_{1,14}=0.3$ , NS). Thus, the data of the two groups were pooled (Fig. 6).

In the first test, bees significantly preferred the novel pattern corresponding to the layout previously rewarded (+ versus -:  $N=16$ ,  $P<0.00005$ ). This indicates that bees were indeed able to extract the common layout of the rewarding stimuli and could transfer this information to the novel test stimuli. This performance thus suggests that bees can categorize patterns on the basis of multiple features. In the second test, bees preferred the mirror image of the rewarded layout to the chequerboard (MI+ versus Ch:  $N=16$ ,  $P<0.05$ ). In the third test, they preferred the left–right transformation of the rewarded layout to the chequerboard (LR+ versus Ch:  $N=16$ ,  $P<0.001$ ). Finally, in the fourth test, no significant difference was found in the choice of left–right transformation and mirror image although there was a marginally nonsignificant tendency to prefer the former (MI+ versus LR+:  $N=16$ ,  $P=0.077$ ).





**Figure 6.** Percentage of choices in the different tests of experiment 2. Under each column the test alternative is shown: MI=mirror image; Ch=chequerboard; LR=left–right transformation. Values are  $\bar{X} \pm SE$  from 16 bees. \* $P < 0.05$ ; \*\* $P < 0.001$ .

### *Discussion*

This experiment indicates that bees can build a generalized representation of the trained patterns on the basis of a common feature configuration. In other words, they are not only able to extract the single orientations contained in each quadrant of a series of different patterns (van Hateren et al., 1990), but can also associate a specific orientation with each retinal quadrant and approach the pattern, provided that a particular quadrant contains a particular orientation (i.e. a summing point that is fed by four independent inputs). Pattern categorization is demonstrated by the fact that bees transferred their choice from the trained stimuli to novel ones sharing the same configuration (first test).

Precluding the formation of a template did not affect the bees' ability to generalize their preference to the mirror image or the left–right transformation of the rewarded layout. As such a layout consisted of a set of four different orientations, the preference of left right

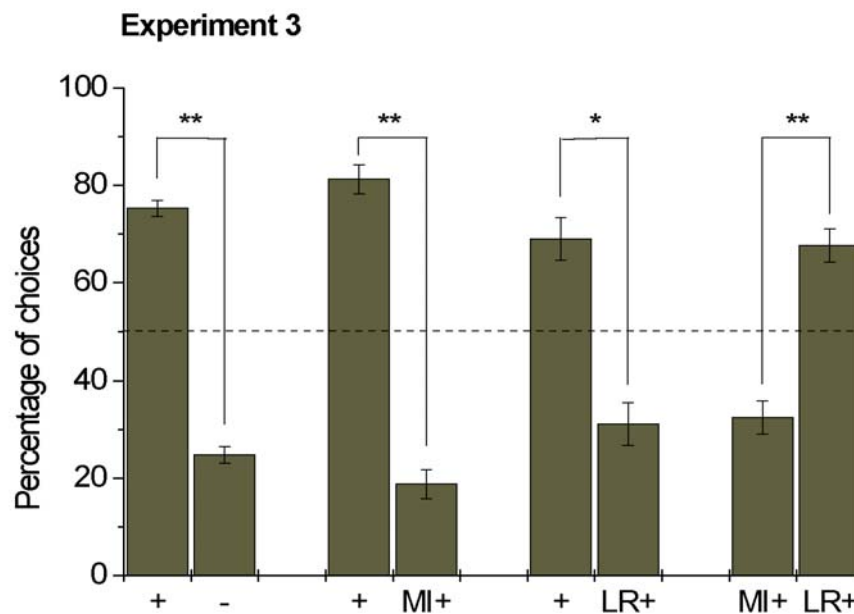
transformation or mirror image to the chequerboard could be based on the fact that the latter did not contain orientation information.

### **Experiment 3**

#### *Results*

No significant differences were found in the performance of the two groups of bees reciprocally trained in this experiment (one group of six bees rewarded on stimuli A1–A6 and nonrewarded on stimuli B1–B6 and another group of seven bees rewarded on stimuli B1–B6 and nonrewarded on stimuli A1–A6:  $F_{1,11}=4.48$ , NS). Thus, the data of these two groups were pooled (Fig. 7).

Bees significantly preferred the novel stimulus that had the configuration of the rewarding stimulus (+ versus -:  $N=13$ ,  $P<0.0005$ ). In the second test, the bees significantly preferred the novel stimulus with the rewarding configuration to its mirror image (+ versus MI+:  $N=13$ ,  $P<0.0005$ ). In the third test, the bees significantly preferred the stimulus with the rewarding configuration to its left–right transformation (+ versus LR+:  $N=13$ ,  $P<0.05$ ). Finally, in the fourth test, the bees significantly preferred the left–right transformation to the mirror image (MI+ versus LR+:  $N=13$ ,  $P<0.0005$ ).

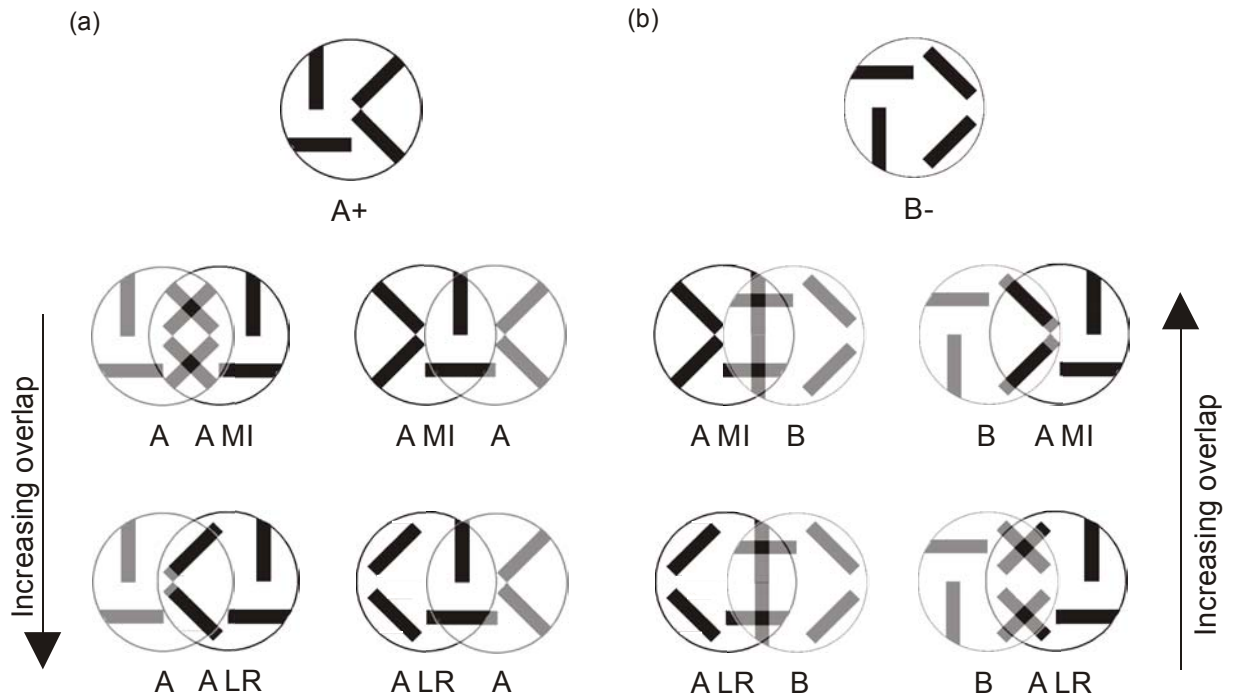


**Figure 7.** Percentage of choices in the different tests of experiment 3. Under each column the test alternative is shown: MI=mirror image; LR=left–right transformation. Values are  $\bar{X} \pm SE$  from 13 bees. \* $P < 0.05$ ; \*\* $P < 0.001$ .

### *Discussion*

Experiment 3 again suggests that bees can extract the common configuration of the rewarded patterns and transfer it to novel stimuli that presented reduced visual information (a single bar) but preserved the original configuration. Furthermore, the choice behaviour of the bees can be explained by assuming that they evaluated the overlap between the extracted configuration and that of the image actually perceived. The learned configuration should be preferred to any transformation, which was indeed the case. Also, the left–right transformation should be preferred to the mirror image of such a generalized configuration because the former yields twice as much overlap with the extracted configuration as the latter (see Fig. 8a). This was indeed the case (Fig. 7). Similar arguments are valid if one considers overlap with the nonreinforced configuration (see Fig. 8b). In this case, the mirror image should be avoided more than the left right transformation, which was indeed

the case (Fig. 7).



**Figure 8.** Recognition based on a spatial arrangement of independent orientations feeding on a common point as an explanation of the results of experiments 2 and 3. The orientation configuration common to the A-type of patterns was taken as rewarded and that common to the B-type of patterns as nonrewarded. The transparent patterns represent the positive (a) and the negative (b) configurations available in memory. (a) The overlap between the positive configuration and its left–right transformation is higher than that between the positive configuration and its mirror image. (b) The overlap between the negative configuration and the left–right transformation of the positive configuration is lower than that between the negative configuration and the mirror image of the positive configuration.

## General Discussion

Our study raised the question of whether bees can transfer the information acquired about a previously rewarded pattern to its mirror image and/or its left–right transformation. Our results show that bees prefer both transformations to a novel stimulus that does not contain orientation information. The transformations were not ranked similarly: bees preferred the left–right transformation of a trained pattern to its mirror image. Two different visual mechanisms may explain the performance of our bees: (1) a low-level mechanism, matching with a retinotopic template of the trained pattern and (2) a higher-level mechanism, matching with an abstracted, generalized representation common to a series of rewarded patterns. Although both strategies are based on comparison between an image currently perceived and an image that has to be accessed from memory contents, they constitute different options as the former has little flexibility while the latter allows for visual categorization of novel patterns.

When only a single pair of training patterns was used (experiment 1), the results can be explained by assuming that bees build a retinotopically fixed template of the rewarded pattern. Such a low-level visual mechanism has been repeatedly suggested in insect visual recognition (Wehner, 1972; Gould, 1985; 1986; Dill et al., 1993; Giurfa et al., 1996a) and involves memorizing a shape in a pixel-based representation that preserves all resolvable spatial details. With such an image in memory, the insects' choice is thought to be determined by the degree of overlapping between the memorized image and the observed shape. The results of experiment 1 are consistent with this possibility. As the single rewarded pattern was kept constant throughout the training procedure, bees could learn it as a template. If this was the case, the choice performance in the different tests of this experiment can be easily explained: the chequerboard was avoided as it offered minimal overlap with the memorized image while, from the two transformations tested, the left–right one offered more overlap with the template of the training pattern than the mirror

image (see Fig. 5), and, accordingly, was preferred. Thus, reference to higher-order recognition mechanisms is not required to explain the bees' preference for the mirror image or left–right transformation of a learned pattern to a novel pattern. The terms mirror image ambiguity or left–right transformation ambiguity as used by Gould (1988), Korneluk & Plowright (1995) and Plowright (1997) could therefore be misleading as they could be associated with some higher-order cognitive phenomena like those operating in the recognition of image transformations by some vertebrates (e.g. mental rotation; see Shepard & Metzler, 1971; Cooper & Shepard, 1973; Cooper, 1975; Cooper & Podgorny, 1976; Hollard & Delius, 1982). The results of the second experiment were based on a different visual recognition strategy as building of a template was impeded by the training procedure. Randomization of pattern presentation is a standard technique that has revealed flexible recognition strategies such as feature identification, that is, recognition of a series of different patterns based on a single common feature. The procedure was inspired in Harlow's (1949) learning sets and has been repeatedly used to study complex visual learning in honeybees (see Giurfa & Lehrer, 2001 for a review). In experiment 2, rather than just a single feature, a spatial configuration of different features, in this case, different orientations, was maintained. By constantly randomizing the training patterns but keeping a common configuration constant, we forced the bees to extract and learn the features common to the patterns experienced sequentially. In that way, bees memorized not the individual patterns but their common features. Such a memory is indicated by the capacity to generalize to novel stimuli presenting such an arrangement of features (i.e. the first test of experiments 2 and 3). As bees correctly transferred their choice to such novel stimuli, their recognition strategy must have been different from one in which a pixel-based template is used.

The capacity to categorize novel patterns evinced in experiments 2 and 3 implies

that bees had built a pattern representation based on the features common to the rewarded patterns. In experiments 2 and 3, we suggest that the pattern representation that bees used when choosing between novel patterns consisted of a set of four different orientations,  $0^\circ$ ,  $90^\circ$ ,  $45^\circ$  and  $135^\circ$ , each in a specific spatial position with respect to the others. Bees would associate a specific orientation with each retinal quadrant and approach the pattern, provided that a particular quadrant contains a particular orientation (i.e. a summing point that is fed by four independent inputs). In both experiments, the stimuli of the first test were new to the bees but one nevertheless preserved the configuration of the rewarded series of patterns and the other that of the nonrewarded patterns. In both cases, bees were capable of choosing correctly the novel stimulus that was coincident with the positive configuration. In experiment 2, both the left–right transformation and the mirror image of the positive configuration were preferred to the chequerboard. This result is not surprising: if the bees learnt the training stimuli on the basis of their common orientation configuration, they should always prefer a pattern containing oriented stripes to the chequerboard that did not contain orientation information. Thus, any transformation should be preferred to the chequerboard, a prediction that was confirmed by our results. Finally, the left–right transformation should be preferred to the mirror image because it overlaps more with the positive orientation configuration. This prediction could not be confirmed in the fourth test of experiment 2, although bees showed a clear tendency to choose the left–right transformation and not the mirror image (Fig. 6).

To address the question of whether the patterns were really learned on the basis of a common configuration in which each quadrant is associated with a different orientation, we performed experiment 3. Here the test patterns contained only a single bar of a specific orientation in each quadrant. The results (Fig. 7) again showed that the bees always chose the test stimulus providing the best match between the actual arrangement of oriented bars

and the memorized positive configuration (see Fig. 8a). This experiment provides clear evidence that bees were not using a single orientation to decide between patterns. If bees had used only one orientation (for instance, horizontal in the lower visual field), they should not distinguish the positive configuration from either its mirror image or its left–right transformation as in both cases horizontal was available in the lower visual field. Furthermore, they could never prefer the left–right transformation to the mirror image as in both cases the horizontal bar was in the same position. Although it is clear that bees did not choose between patterns on the basis of a single orientation, they might associate different orientations with the top and the bottom halves of the patterns. They would look for particular orientations at the top of their retina and for other orientations at the bottom. This possibility is consistent with our suggestion about how bees would try to match the memorized pattern with the currently perceived one: they would scan the perceived pattern in the horizontal direction (see Figs 5 and 8) and not in the vertical one. It is a common observation that bees flying within a maze maintain a constant height in the apparatus but exhibit a high flight activity in the horizontal plane.

Object visual recognition by humans (see Wallis & Bülthoff, 1999 for a review) also admits the use of a holistic, template-based recognition strategy and of a feature-based recognition strategy. The feature-based recognition theories in humans propose that a pattern consists of a set of specific features or attributes. The process of pattern recognition is assumed to begin with the extraction of the different features from the stimulus presented, which are then combined and compared against information stored in memory. This option is different from the feature-based recognition theory in insects, in which it is assumed that a single feature is used for recognition. Our work indicates that extraction and combination of multiple features could also be a possibility in insect visual recognition.



Recognition based on a set of independent features with a common summing point is different from template-based and single feature-based recognition strategies, the two options acknowledged up to now in insect vision. Matching with a retinotopic template implies a memory of a pattern on a pixel basis, a capacity repeatedly suggested in insects (Wehner, 1972; Gould, 1985; 1986; Dill et al., 1993; Giurfa et al., 1996a). Feature extraction, on the other hand, implies selective attention for a single feature common to different rewarded patterns. In this case, insects extract the reinforced feature from multiple exposures to such patterns and can later generalize their choice to novel patterns presenting the reinforced feature. Thus, they can categorize patterns on the basis of a single reinforced feature (van Hateren et al., 1990; Lehrer et al., 1990; Horridge & Zhang, 1994; Giurfa et al., 1996b). Thus, our results suggest that recognition can be based on more than one feature. This possibility represents a further step in complexity compared to single-feature recognition.

So far, we have only mentioned the possibility that bees learn the configuration common to the rewarded stimuli in an excitatory way; but they may also learn the configuration common to the nonrewarded stimuli in an inhibitory way. In a differential conditioning procedure like ours bees learn not only to choose the rewarding patterns but also to avoid the nonrewarding ones (Giurfa et al., 1999). If we assume that bees extracted and learned not only the positive configuration of the rewarded stimuli but also the negative configuration of the nonrewarded ones, the choice of bees in experiments 2 and 3 becomes clearer. The left–right transformation offers not only more overlap with the positive configuration than the mirror image but also no overlap with the negative configuration. The mirror image, on the other hand, overlaps on one side of the negative configuration (see Fig. 8b). Thus, the left–right transformation should be preferred to the mirror image, not only because it overlaps more with the rewarding configuration, but also

because it is less inhibitory than the mirror image.

Srinivasan (1994), Srinivasan et al. (1994) and Zhang & Srinivasan (1994) have suggested that three sets of orientation detectors with coarse angular tuning with axes at  $120^\circ$  to each other are necessary to describe orientation discrimination in honeybees. Furthermore, by mean of electrophysiological experiments, Yang & Maddess (1997) found such orientation detectors in the lobula, one of the visual neuropiles of the bee brain. Such detectors, however, cannot be used to discriminate the patterns we used as they have large visual fields extending over the whole eye (Yang & Maddess, 1997). The different orientations of our patterns would be averaged across a large visual field and the specific spatial configuration of orientations would be lost. Orientation detectors with smaller visual fields are required for the implementation of an orientation configuration like the one suggested by our experiments. Such detectors have been found in the medulla of the locust *Locusta migratoria* (Osorio, 1987; James & Osorio, 1996), another visual neuropile of the insect brain. Two main classes of neurons identified there form numerous overlapping retinotopic arrays with receptive fields from  $2$  to  $20^\circ$ . The response of these cells allows us to characterize them as edge detectors. It is thus conceivable not only that they respond to specific orientations but also that their simultaneous activation provides the basis for pattern representation in terms of a specific set of orientations.

Our results are ecologically relevant. As mentioned in the Introduction, in a natural environment the shape of flowers is not always fully visible. It is therefore important that pattern recognition is flexible enough to allow generalization to an incomplete image of the correct flower species. The two basic strategies suggested by our experiments, template-based recognition (experiment 1) and recognition based on a set of independent features feeding on a common point (experiments 2 and 3), allow the bee to solve the problem. One could argue that multiple exposure to patterns sharing a common configuration is an

unrealistic training procedure that would be rarely experienced in natural situations. Actually, the opposite is true. During a single foraging bout, bees visit hundred of flowers of the same species (Winston, 1987). Individual corollas are not identical: genetic variability produces small variations around a common floral display. In this context, extracting and combining the features common to the reinforced display constitutes a valid strategy to ensure flower constancy.

We thus conclude that bees can transfer the information acquired about a previously rewarded pattern to its mirror image and/or its left–right transformation. However, one has to be cautious in defining the cognitive implications of these results. The fact that parsimonious explanations are possible based on low-level visual recognition strategies, and not on higher-level mental operations such as those postulated for mirror image recognition in humans, indicates that insects may have different ways to solve such recognition problems. In that sense, focusing on the mechanisms by which humans and other animals solve the same problems can be enlightening.

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