



Evidence against a retinotopic-template matching in honeybees' pattern recognition

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Abstract

Currently two hypotheses exist as to how insects process visual images, as photograph-like 'retinotopic-templates', or as a set of features extracted by the visual system. Several results obtained in honeybees cannot be reconciled with a retinotopic-template matching. (i) Bees discriminated between two patterns that should not be distinguished according to the template hypothesis. (ii) Bees preferred patterns that showed no overlap with the assumed template to patterns that had such an overlap. (iii) Bees showed a generalization of properties of the rewarded pattern to other patterns. Thus, in our paradigm, the bees must have used additional mechanisms and cues for the processing and classification of patterns. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Since the first investigations on honeybees (von Frisch, 1915; Hertz, 1929, 1933) a plethora of studies has been devoted to the question of what kind of processing strategies enable insects to recognize and classify visual patterns (for reviews see Wehner, 1981; Srinivasan, 1994; Ronacher, 1998). Two broad classes of concepts can be distilled from these studies. (i) The 'feature' or 'parameter' hypothesis assumes that bees — like other animals — detect some characteristic features of objects, and that recognition is based on a comparison of actual parameter values with the previously stored values of features like size, contrast, contour density, presence of oriented contours, of points, and many others (e.g. Schnetter, 1968; Anderson, 1977a; Ronacher, 1979, 1998; Wehner, 1981 van Hateren, Srinivasan, & Wait, 1990 Srinivasan, 1994; Giger & Srinivasan, 1995; Giurfa, Eichmann, & Menzel, 1996a). (ii) The 'template hypothesis' assumes that

a template of the learned pattern is stored in a way that preserves the retinotopic coordinates, and that recognition depends on the evaluation of the overlap between an actual retinal image and the stored template. This idea of a 'retinotopic-template matching' was first put forward for honeybees' pattern recognition by Wehner (1969, 1972)(cf. also Cruse, 1972, 1974) and has later been termed 'eidetic template' hypothesis by Gould (1985, 1986) (for reviews see Srinivasan, 1994 Heisenberg, 1995; Lehrer, 1997). The two hypotheses differ substantially in assuming different amounts of preprocessing prior to the storage of information about a pattern. Furthermore, the parameter hypothesis is in accord with invariance mechanisms while invariances are not expected on the basis of the retinotopic-template hypothesis.

Two predictions can be derived from the template hypothesis, (i) there should be little generalization to other (e.g. rotated or shifted) patterns, and (ii) the classification of patterns will depend on the amount of overlap between an actual image and the template rather than on the presence of special feature elements or fine details of a pattern. These predictions were indeed met in experiments with tetheredly flying *Drosophila*. The flies did not recognize a previously reinforced pattern if this pattern was subjected to a

Abbreviations: TR +, rewarded training shape; TR -, unrewarded training shape.

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minute (vertical) shift in retinal coordinates (Dill, Wolf, & Heisenberg, 1993). In addition, the discrimination of patterns appeared to depend largely on the area of overlap with the trained pattern, strongly suggesting a template mechanism (Dill & Heisenberg, 1995). Conceivably, this type of processing may have been favored by the specific training and test situation the animals experienced in the flight simulator. Indeed, recent experiments yielded evidence that flies may perform a feature extraction even in the situation of the flight compensator (Ernst & Heisenberg, 1999).

Similarly, the upshot of several recent studies in honeybees is that both processing mechanisms seem to coexist also in this species, and might be differently applied depending on the task in question (Heisenberg, 1995; Giger & Srinivasan, 1995; Ronacher & Duft, 1996). In an earlier experiment (Ronacher & Duft, 1996) the bulk of the results was compatible with a template matching, but a feature extraction could not be excluded (cf. also Heisenberg, 1995). We now wanted to put the template hypothesis to a critical test in a similar training paradigm as before (patterns presented on a vertical screen). By systematically varying the degrees of overlap of test patterns with the postulated template we now obtained several results that are incompatible with the template hypothesis. In addition, our results indicate that the bees may use several different features for the classification of patterns.

2. Methods

2.1. Apparatus

Single, freely flying bees (*Apis mellifera carnica*) were trained on a pair of patterns presented behind a vertical perspex plane. The apparatus consisted of a cube of perspex plates mounted on a turntable (Fig. 1). This cube allowed the training and testing procedures to be carried out on each of the four sides (each measuring $64 \times 64 \text{ cm}^2$). For a visiting bee only one side was visible, the others were concealed behind white cardboard. In all other respects, however, the bees were not restrained in their approach towards the apparatus. In the centre of each plane there were two entry holes (25 cm apart from each other), through which the bee could enter a tube (1.5 cm outer diameter). Inside the tube of the positive training shape (TR+), the bee found a small reward of sugar water, which had been deposited by the experimenter via a syringe or via a pump from behind. The tube of the negative training pattern (TR-) remained empty. The black-and-white patterns were mounted on a disc of perspex (10 cm radius) by which the pattern was pressed from behind against the perspex plane in front. The background (6.5 cm behind the front plane) was covered with white cardboard. The perspex plane was illuminated by two 120 W tungsten lamps (light intensity approximately 13403 lux) in a way that minimized shadows and reflexions. To reduce possible influences of scent, two sides of the cube were used only for training, and the two remaining sides were used exclusively for tests. In addition, the perspex front plates and the reward tubes were washed with 40% ethanol after each visit of the bee and frequently exchanged, in order to exclude any influence of scent based orientation.

The bees were not restrained in their approach to the apparatus, and could freely adjust their distance before deciding for one of the patterns. Thus, this experimental set-up did not control for the image size at the bee's decision point. This possible disadvantage, however, had to be accepted since there are reports that an experimentally enforced decision from a larger distance would preclude the formation of a template, and prompt the animals to a feature extraction (Lehrer, 1993; Srinivasan, Zhang, & Witney, 1994; Giger & Srinivasan, 1995; Horridge, 1996). Therefore, if one wants to test the template matching hypothesis in bees, the animals should be allowed to inspect the patterns from close distances (cf. also Wehner, 1969, 1972).

2.2. Training and test stimuli

Three different training paradigms were used with different bees (compare insets on top of Figs. 2 and 5). During training A the TR+ was a large equilateral

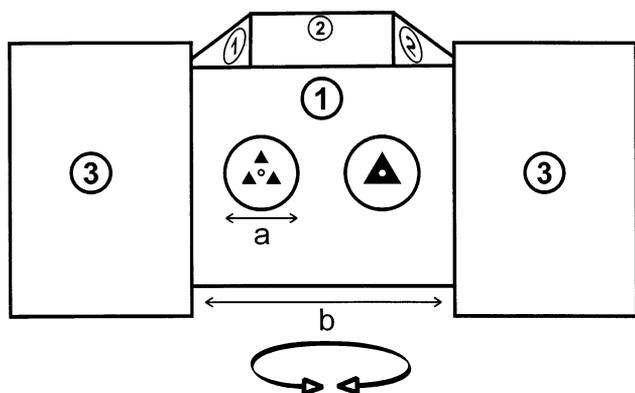


Fig. 1. Scheme of the apparatus used for training and tests. The apparatus was a perspex-cube whose faces could be easily removed. For reasons of clarity, the background of white cardboard, which was situated 6.5 cm behind the front plate, is not indicated in the drawing. The cube was mounted on a turntable, so that each of the four faces of the cube could be presented to the bee. The access to the other faces, however, was prevented by means of white cardboard covers (3). Two faces of the cube (1) were used exclusively for training, the other two (2) for tests. The patterns were pressed from behind against the perspex front plate ($64 \times 64 \text{ cm}^2$) by means of a 10 cm (radius) perspex plate.

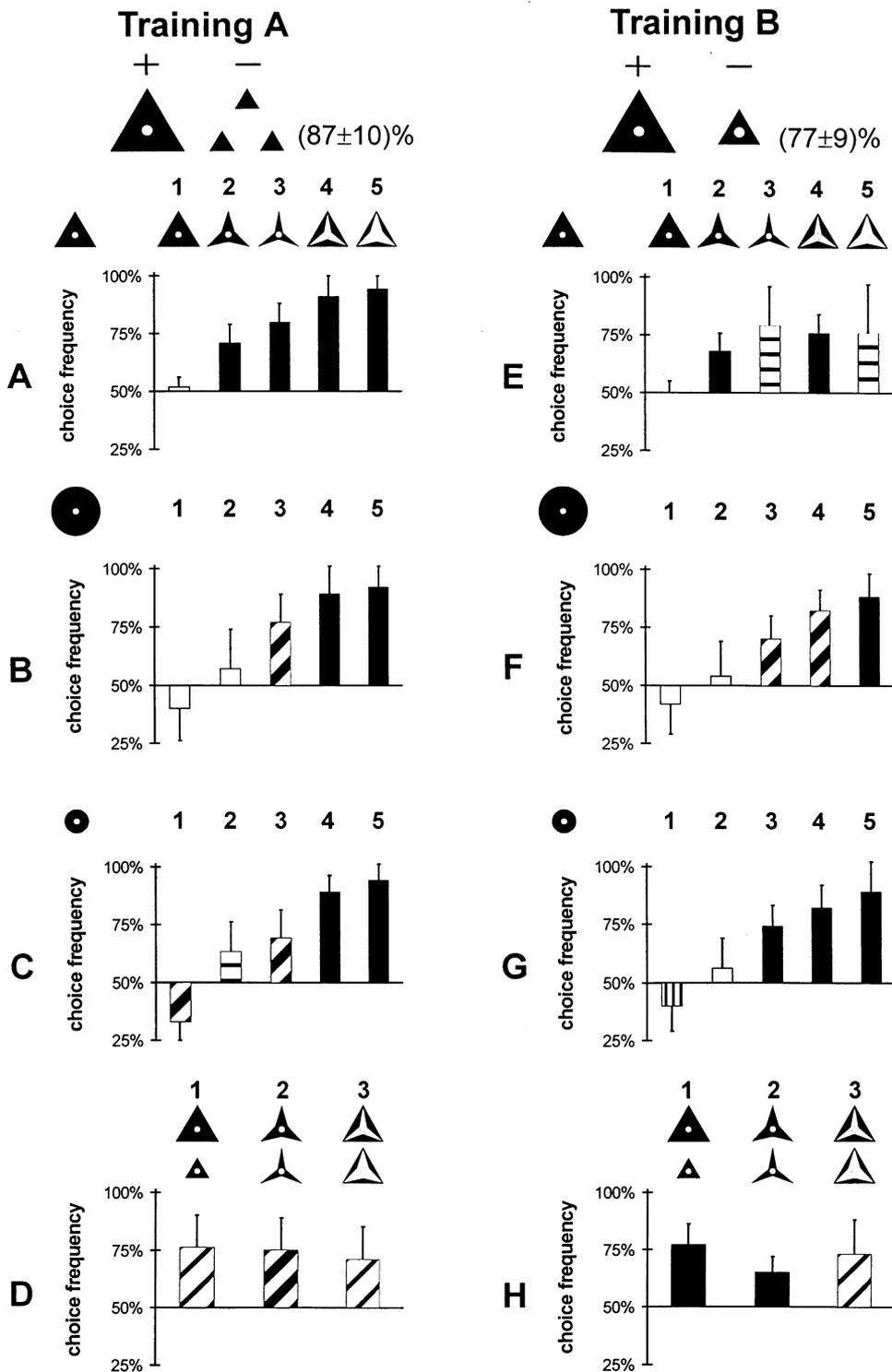


Fig. 2. Classification of different patterns by bees having participated in training paradigm A (left column) or B (right column). All tests were performed as pairwise comparisons; pictograms of one of the test patterns are shown above the columns (which are also numbered to facilitate referencing in the text), the other test pattern, which was held constant for a test series is shown on the left of each diagram. Choice frequencies are calculated for the pattern shown on the left (or, in case of D and H, to the upper of the two patterns). Thus, e.g. in C a choice frequency above 50% indicates that the small disk was preferred. Bars above the columns indicate S.D., based on the results of at least $N = 7-9$ individual bees in each case. In this and the following figures the significance levels (tested against a random, 50:50 choice) are indicated by the texture of columns, black $P < 0.001$; narrow oblique stripes $P < 0.002$; wide oblique $P < 0.01$; horizontal stripes $P < 0.02$, vertical stripes $P < 0.05$; open columns, n.s.

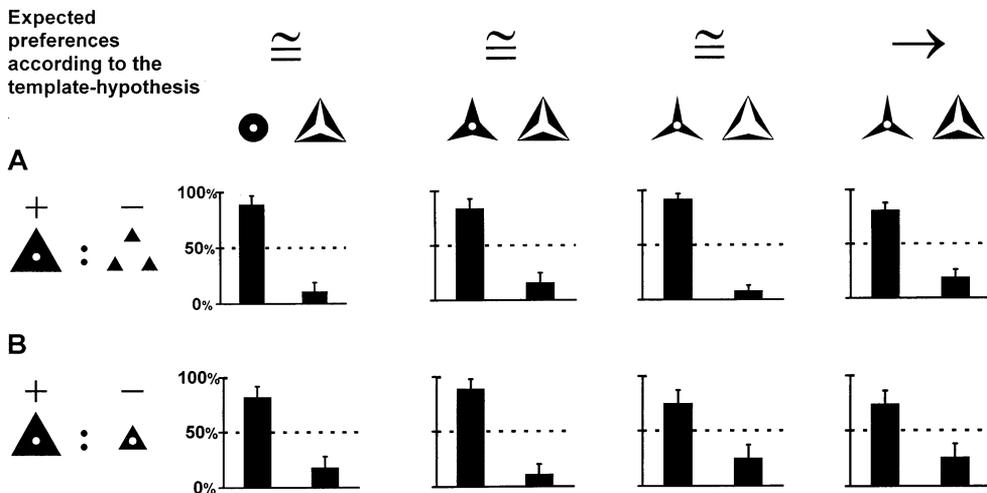


Fig. 3. Is discrimination of patterns based on their overlap with a template? Tests of patterns exhibiting approximately the same overlap area with the TR + (columns 1–3) or with a 1/3:2/3 overlap (column 4). The expected preferences according to the template hypothesis are indicated above the patterns. Upper row, training paradigm A, lower row, training paradigm B (tests were performed with different groups of bees!). In each pair, the left of the two patterns was significantly preferred ($P < 0.001$; $N = 7-8$).

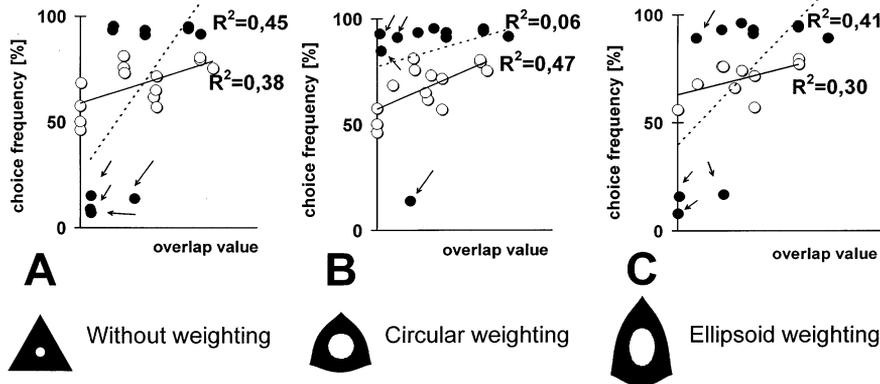


Fig. 4. Tests for a possible influence on template matching of a distorted pattern projection upon the compound eye. Data from training A (e.g. Figs. 2 and 3). (A) Choice frequencies (ordinate) for a given test pair plotted against the difference between the respective overlap value with the TR + of each of the two test patterns (abscissa). Choice frequencies were calculated for the pattern with larger overlap to TR +. (B) Overlap values were calculated after a weighting that compensates for the distorted projection of central and peripheral parts of a pattern (at an assumed distance of 3 cm between bee and pattern, cf. Section 2); same data set as in A. (C) Ellipsoid weighting function (3 cm distance). Open symbols, test pairs in which both patterns had the same ‘color’ in their centre; closed symbols, the two patterns of a test pair differed in their respective centres (cf. Fig. 3). See text for further details.

black triangle (pointing upwards) and the TR – was a pattern composed of three small triangles, arranged in the corners of the large triangle (see Fig. 2). In training B, the TR + was the same triangle as in training A, while the TR – was a small black triangle located in the centre. Training C was the reciprocal to training A: now the TR + consisted of three small triangles and the TR – was the large black triangle (see inset on top of Fig. 5). The two reciprocal training situations were intended to check for spontaneous pattern preferences of the bees.

All training and test patterns (see insets in Figs. 2–6 and 8) consisted of black-and-white photocopies of high contrast. The sides of the equilateral triangle used

as TR + in training A and B were 100 mm long. The small triangle used as TR – in training B had a side length of 57 mm and its area was one third of that of TR +. The three small triangles used as TR – in training A (TR + in training C) had each a side length of 33 mm and an area, which was one ninth of that of the large triangle. The stars and open triangles used had a black area of either approximately two thirds or one third of the large triangle (Fig. 2). In addition, discs of 58 or 29 mm radius were used. The large disc circumscribed the 100 mm triangle (circumcircle), the small one was completely contained within the triangle. All test patterns used here were centred around the reward tubes.

2.3. Training and test procedures

During training a single, individually marked bee had to visit, through a slit in the window, the experimental room, in which the apparatus was located. The illumination of the apparatus by daylight was prevented by means of a shutter. During discrimination training the bee found a reward of 20–40% sucrose solution in the tube connected to the centre of the positive training pattern (hereafter referred to as TR +). It did not find any reward within the tube of the TR – stimulus. The amount of reward was adjusted so that the bee had to choose TR + between 5 and 15 times during a visit (i.e. before returning to the hive), and the concentration of the sugar solution was adjusted so that the experimental bee did not attract many follower bees. During training the left–right position of the rewarded stimulus was changed between every two to three visits, in order to prevent the bee from establishing side preferences. As soon as the bee had reached a stable discrimination level (for training A and C > 80%, for training B > 70%), which usually occurred after 15–20 visits (corresponding to 1–2 h of training) the test procedure began. All tests were performed as pairwise comparisons without reward. At the end of a 2–6-min test period, however, the apparatus was turned as to reveal the training pair, and the bee received a drop of sucrose solution on TR + . Different test pairs were presented in a quasi-random order, which was different for individual bees; between any two tests bees performed at least two to four training visits. The learning level was frequently controlled by tests, in which TR + and TR – were presented (without reward). Tests with two identical patterns were occasionally interspersed in order to check for side preferences (see test no. 1 in Fig. 2A and E). Further, in successive tests the two patterns of a given test pair were presented in different positions (left and right), in order to compensate for remaining small side preferences. The bee was regarded as having made a choice when it had actually landed on a pattern, i.e. the bee had touched the pattern or the entrance of the central tube with its legs. For each test pair the choice frequency was calculated according to $CF = x \times 100 / (x + y)$ with x and y representing the number of decisions of an individual bee for pattern X and Y , respectively. Usually each choice frequency value of an individual is based on 15–25 landing decisions. Tests for which less than ten decisions could be obtained were excluded from the data analysis.

Mean values and standard deviations, which are presented in the figures are derived from the choice frequencies of individual bees. In most cases the mean values are based on the individual choice frequencies from at least seven to eight bees (exceptions, Fig. 6C,

Fig. 8B). Statistical significance of the choice frequencies (compared with a 50:50 random choice) was tested by a t -test with $N - 1$ degrees of freedom ($N =$ number of bees tested). If choice frequencies exceeded 70%, the data were arcsine transformed before applying the t -test (Sachs, 1997).

When a bee sees a pattern from close distance, the central parts of the pattern will be seen under larger visual angles than the peripheral ones, due to the spherical design of the compound eye. For example, at 3 cm viewing distance, a pattern element of 1 cm length covers an angle of 19° when viewed frontally, but only 6° when located 5 cm off axis. This distorted projection of lateral pattern elements could influence our calculations, if the bees indeed determine overlap values between actual patterns and a stored template. In order to check for a possible influence of a distorted projection of the patterns upon the bee's eyes, we used the following weighting procedure: each pattern was superimposed with a mask of equidistant (0.5 cm) concentric rings and the amount of black area of the pattern falling within each ring of the mask was determined. Then the visual angles under which each ring appeared (from an assumed viewing distance of 3 cm) were determined and the corresponding pattern area was weighted with this value according to the formula

$$\alpha = \arctan\left(\frac{r_a}{d_v}\right) - \arctan\left(\frac{r_i}{d_v}\right)$$

where α is viewing angle, d_v the viewing distance (here 3 cm), r_a and r_i are the distance from the centre of outer and inner border of a ring.

These weighted pattern areas were then used to calculate the respective overlap values with TR + for each pattern. As a first rough approximation, this procedure was performed for a mask with radially symmetric rings, considering the bee's compound eye as a regular sphere. In order to adopt a more realistic picture we also used a mask of ellipsoids (with a 2:1 ratio of the main axes; cf. pictograms in Fig. 4B and C). In Fig. 4 the difference between the two overlap values of a pair of patterns (with the template of TR +) is plotted on the abscissa (see Ronacher & Duft, 1996, for a discussion of other 'similarity' functions).

The flight path of a trained bee was video recorded during tests, and analyzed off-line frame-by-frame. As an example, we evaluated tests in which the bees had to discriminate the TR + (of training C) from the inverted TR + (Fig. 7). A grid was superimposed over the picture of each stimulus, and the movements of bees recorded by measuring the number of times each square was crossed (Anderson, 1977b). The frequency of visits to different regions of the pattern (mean number of square crossing per bee) is indicated by different shades of grey in Fig. 7.

3. Results

3.1. Did the bees learn the triangular shape of TR + ?

In the first test series, the bee had a choice between combinations of patterns that showed stepwise modifications of TR + (the large black triangle). Stars and triangles with white centres, thereafter called ‘open triangles’, were tested against the TR +, or a black disc. For each pattern type two degrees of overlap with TR + (2/3 and 1/3) were tested (cf. insets in Fig. 2A). In training paradigm A, the bees discriminated significantly between the black triangle and both stars, and, with even higher choice frequencies, between the black and open triangles. Although the area of overlap with TR + was the same (two thirds) for the patterns shown in column 2 and 4 (likewise for columns 3 and 5, one third), the differences between the choice frequencies for tests nos. 2 and 4, and for test nos. 3 and 5 in Fig. 2A are significant at $P < 0.01$. Unexpectedly, the bees did not discriminate between the triangle and a large black disc (circumcircle, test no. 1 in Fig. 2B) and showed only a weak preference for the triangle when tested against the small disc (test no. 1, Fig. 2C). Both types of disc were preferred against stars and open triangles (Fig. 2B and C; all but one choice frequency values being significantly different from a 50:50 choice).

Obviously, in this training paradigm, the bees did not rely on the shape, i.e. the triangular outline of the TR +. This is particularly evident in Fig. 2C, the small disc was uniformly preferred although the stars had acute points at the correct position, and the open triangles exhibited acute points and straight contours at the correct positions, thus sharing a number of characteristic features with the rewarded triangle. From the choice behavior depicted in Fig. 2B and C we have to conclude that for the bees it was the disc rather than the open triangle, which was more similar to the rewarded triangle.

3.2. Overlap with TR + cannot be the decisive cue

The results of the tests shown in Fig. 2D, (and some of the data in Fig. 2A–C) could be interpreted as indicating a correlation between choice frequencies and degree of overlap with TR + of a particular test pattern. As a critical test for this possible correlation, bees were now given a choice between pairs of patterns that both had roughly the same overlap with TR + but differed distinctly in other respects (one exhibiting a black, the other a white centre, Fig. 3A, columns 1–3). In the rightmost column of Fig. 3A, a combination is shown in which the overlaps with TR + of star and open triangle were 1/3 and 2/3, respectively. The symbols above the pattern combinations indicate the expected preferences, if bees classified the patterns

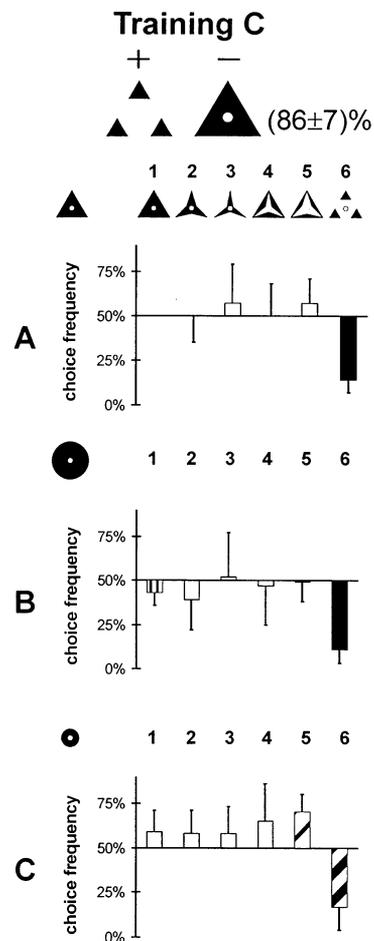


Fig. 5. Classification of different patterns by bees trained in paradigm C. Same notation as in Fig. 2. $N = 7-8$ bees tested for each data point. Significance values are indicated by the texture of the columns, see legend of Fig. 2.

according to their degree of overlap with TR +. As becomes evident from Fig. 3A, the bees definitely ignored these expectations, in all tests they showed a strong preference for the pattern that exhibited a more or less compact black central area. This preference held even in case that the black-centred pattern had a much smaller overlap with TR + (Fig. 3A, rightmost column, respective overlap values 1/3 and 2/3).

3.3. Training paradigm B: a control for a possible avoidance of TR –

It is conceivable that the choice behavior visible in Fig. 3A, as well as the generally high choice frequencies for the pattern combinations including an open triangle in Fig. 2, could have been due to a learnt avoidance of patterns with a white centre, which had been induced by the particular shape of TR – in training A. In order to check for this possible explanation of the results of Fig. 3A, we repeated these tests with a different group of bees that had been trained with paradigm B (Fig.

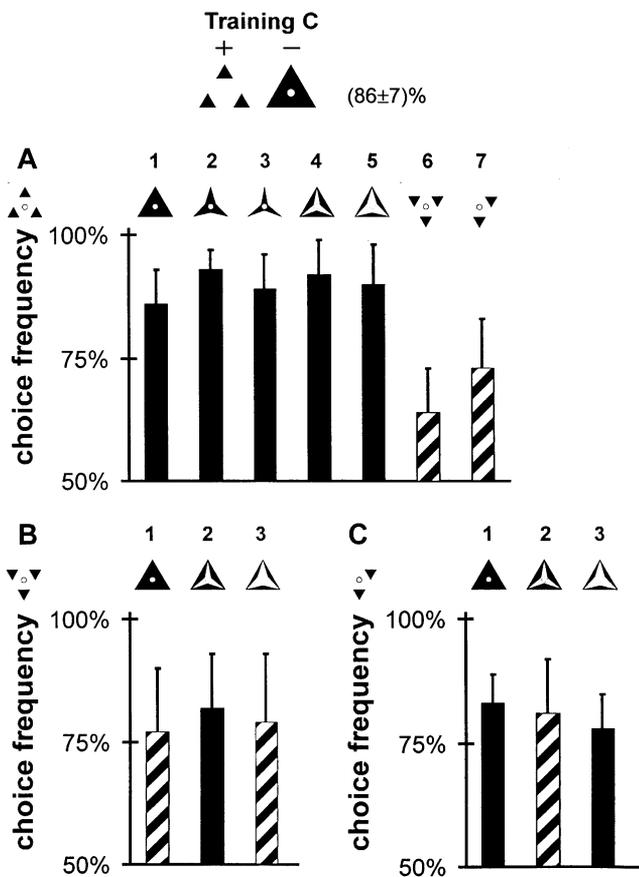


Fig. 6. Classification of different patterns by bees trained in paradigm C. Same notation as in Fig. 1. $N = 7-8$ bees tested for each column of A, B ($N = 5-6$ bees for C). Significance values are indicated by the texture of the columns, see legend of Fig. 2. Note that the rotated version of TR + shown in B has no overlap with the assumed template of TR + if the reward tube is taken as point of reference; cf. also Fig. 7.

3B). In this case both, the rewarded and the unrewarded, training patterns had a black centre and differed only in the periphery of the patterns, thus offering no incentive for avoiding white-centred patterns. The discrimination level was somewhat lower for this training pair, and in general also the mean choice frequency levels obtained in the tests of Fig. 3B were lower compared with those of Fig. 3A. Nonetheless, the data shown in Fig. 3B demonstrate a clear preference for the black-centred patterns, irrespective of the overlap areas. These results refute the assumption that an acquired avoidance of open patterns, which was due to the shape of TR – in training A, was responsible for the results of Fig. 3A. In the other tests with training B (Fig. 2E–H) the bees' responses were also highly similar to those of bees experiencing training A. The strong similarity between the choice behavior observed in the two training paradigms indicates that the bees had relied mainly on

properties of the rewarded training pattern in order to identify the food source.

3.4. Influence of the projection of images upon the compound eye

The results of Figs. 2 and 3 suggest that the central region of the test patterns exerted a special impact on the bees' choice behavior. As a next step, we therefore asked whether a preference for the central area might have been caused by the distorted projection of patterns upon the bee's compound eye. As a very rough first approximation, we assumed that the compound eye could be seen as a regular sphere. Depending on the objects' distance, the projection of the central parts of a pattern at the retina is larger than that of the more peripheral parts of patterns (cf. Section 2; see also pictograms in Fig. 4B and C). In Fig. 4 the choice frequencies for different pattern pairs are plotted against the difference in their respective overlaps with TR + (only results from training A are included; choice frequencies are calculated for that pattern of a pair that had the larger overlap with TR +).

We divided the test patterns into two classes. One class (open symbols) contained pattern pairs with similar centres (e.g. triangles against stars, cf. Fig. 2). The other class (filled symbols) consisted of pairs that differed in the central area (e.g. the combinations shown in Fig. 3). The diagram in Fig. 4A shows the results before application of a weighting function to the central and peripheral parts of a pattern. There is a weak positive correlation for the open symbols ($r = 0.61$; $P < 0.05$). The distribution of filled symbols in this kind of diagram, however, indicates conflicting evidence. Several data points lie below 20% choice frequency, indicating that the bees indeed had a strong preference for the pattern with smaller overlap to TR + (Fig. 4A, arrows)! However, if the central area of a pattern is overemphasized due to the distorting projection upon the eye, a better correlation between the choice frequencies and the overlap differences might result. Fig. 4B shows the results after a weighting (according to the visual angles under which different parts of the pattern are seen, for an assumed distance of 3 cm between bee and pattern) has been applied. While for the open symbols this weighting function does not change the correlation by much it distinctly influenced the distribution of filled symbols. Nonetheless, it would be rash to conclude that this procedure substantially improves the correlation between choice frequencies and overlap differences. There is still one data point lying distinctly below 50% (arrow, corresponding to Fig. 3A, rightmost column), indicating a 'wrong' choice of the bees. For the other points marked with arrows the difference in overlap had changed its sign by application of this weighting function. However, this

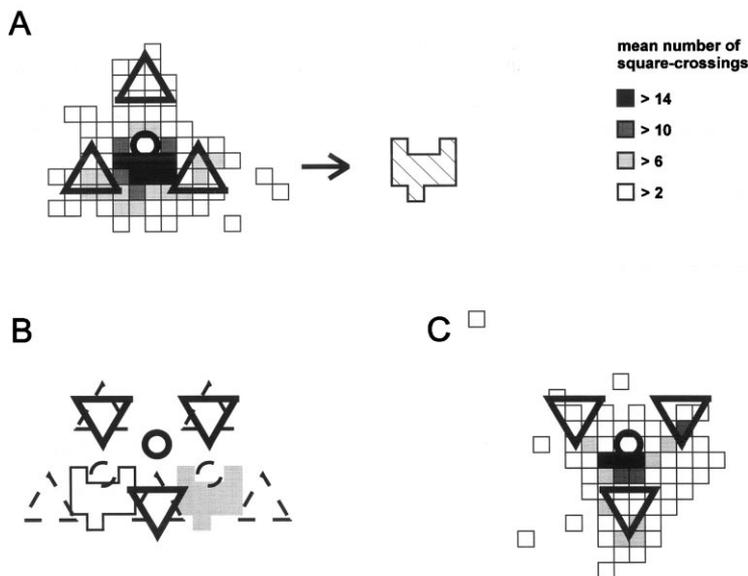


Fig. 7. Video analysis of flight behavior in front of TR + and the rotated shape of TR + (training C). Videotapes were analyzed frame-by-frame, by means of a superimposed grid. The frequency of the bee's crossing the area of a pixel is shown as dark shading of the pixels (crossings and landings on the reward tube have been omitted for reasons of clarity). (A) Shows the search distribution in front of TR +. The centre of the ($N=9$) bees' locations is situated a bit below the reward tube, in an elongated rectangle (see arrow for the extracted pattern of pixels with more than ten crossings). If the bees had tried to match two of the triangles of the rotated TR + with the supposed template, one would have expected a distribution that is shifted to one or the other side (as indicated in B by the outline and the grey areas). (C) The actual distribution of flight paths in front of the rotated TR + differs clearly from the expectation shown in B.

does not really speak in favor of an evaluation of overlap areas, since now we obtain very high choice frequencies (around 90%) for pairs of stimuli that showed only a negligible difference in overlap values (arrows in Fig. 4B). Applying a weighting function based on ellipsoids did not improve the situation (Fig. 4C). The conclusion is that an evaluation of overlap areas does not yield a satisfying description of the bees choice behavior, even if one takes into account the geometry of image formation on the compound eye (Giurfa, Vorobyev, Kevan, & Menzel, 1996b).

3.5. Did the bees 'focus' on the central region of the patterns?

Several of the results presented so far seem to indicate that the bees classified and recognized a pattern mainly on the basis of its central regions — which may have been induced by the special training situation in which the reward was offered in the pattern's centre. This assumption implies a clear prediction for the training paradigm C, which is the reciprocal training to A (see top of Fig. 5), the bees should now exhibit a preference for patterns with white centres.

We repeated the tests of Fig. 2 with a different group of bees that were trained with the training combination C (Fig. 5). The TR + pattern was preferred against the black discs with similar choice frequencies as against the black triangle (i.e. TR -; compare tests nos. 6 in

Fig. 5 A–C). However, for most of the other test pairs the choice frequencies did not significantly deviate from a 50:50 choice. This was true even in the case where an open triangle (with white centre) was tested against the black triangle or a large black disc (tests nos. 4, 5 in Fig. 5 A and B). The small disc was even preferred to one of the open triangles (test no. 5 in Fig. 5C), again contrary to the above prediction. Interestingly, in training C the TR + was discriminated from the open triangles with similar choice frequencies as from the TR - (black triangle) or the stars (Fig. 6A). The good discrimination of TR + and a different pattern with white centre (tests nos. 4, 5 in Fig. 6A), as well as the results of Fig. 5 (tests nos. 4, 5), demonstrate that the presence of a white centre alone was not sufficient to produce the observed classifications of patterns in this training. In addition, the results of Fig. 5A confirm that there was no specific avoidance of TR -, a conclusion that has been derived already from the two other training paradigms.

3.6. Critical tests for the template matching hypothesis

The results of Fig. 5C are of special interest in view of the template hypothesis. The small disc had no overlap with the TR + of training C, while the alternative patterns all overlapped, at least to some degree, with TR +. The data of Fig. 5C give no indication for the expected preference for the stars or open triangles.

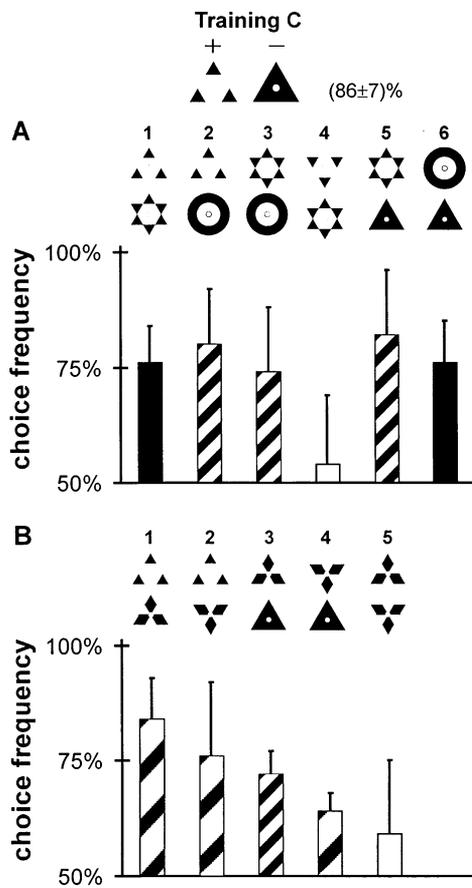


Fig. 8. Classification of different patterns by bees trained in paradigm C. Same notation as in Fig. 1; choice frequencies are calculated for the upper pattern of each pair. (A) $N=7-9$ bees tested per pattern pair. (B) Note that the lower significance levels are due to a smaller number of bees tested, $N=4$ bees for tests nos. 2, 3, 5, and $N=3$ bees for tests nos. 1 and 4. Significance values are indicated by the texture of the columns, see legend of Fig. 2.

On the contrary, in general there was a bias towards the small disc while the template hypothesis predicts a preference for the alternative patterns. Taking a very conservative value of a low 65% — preference for the stars and open triangles, all the choice frequencies in Fig. 5C (tests nos. 1–5) deviate significantly ($P < 0.01$) from this expectation.

In this training paradigm C, we subjected the matching hypothesis to a second test, by using a rotated version of TR+ (Fig. 6). In the direct comparison of TR+ and its rotated version the bees showed a small but consistent preference for the TR+ pattern in its normal position (Fig. 6A, test no. 6, $t=4.01$, $N=7$, $P < 0.01$), which shows that the bees were able to discriminate between the two different pattern orientations. However, if the rotated version of TR+ was tested against the black or the open triangles (Fig. 6B, tests nos. 1–3), it was significantly preferred, although the preferred pattern had no overlap with TR+ while

the alternative patterns all showed some overlap with TR+. Omitting one of the small triangles of the inverted TR+ made the pattern less attractive than TR+ (Fig. 6A, test no. 7). But this reduced pattern was still preferred against the large black triangle and the open triangles (Fig. 6C).

Before we conclude that the results of Fig. 6B and C demonstrate a generalization capacity of the bee, a point of caution has to be raised. It is conceivable that the bees tried to attain a partial match between e.g. two of the small triangles of the inverted TR+ and the template of TR+ (cf. test no. 6 in Fig. 6A). In this case, one would have expected a sideways shift in the distribution of approaches towards the inverted test pattern. A video analysis of the flight behavior of bees in this test, however, did not reveal any indication for a distribution of the bees' locations that had been shifted in the expected way (compare Fig. 7B and C). A similar cluster of dark pixels as for TR+ is found below the reward tube of the rotated TR+, in addition, the bees visited also the small triangles at the lower and upper right position. However, there was no indication for a preference of the positions indicated in Fig. 7B. Furthermore, it should be emphasized that the preference for the disk in Fig. 5C cannot be explained by the bees trying to attain a partial match to the TR+ template. Thus, the results of Fig. 6B and C indicate a generalization.

Additional examples for a generalization capability are shown in Fig. 8. A six-pointed pattern, i.e. a combination of the TR+ and its rotated version, was distinctly less attractive than TR+ (test no. 1 in Fig. 8A). Obviously, the addition of black areas to TR+ made the two patterns distinguishable (cf. Dill and Heisenberg, 1995). Although the bees classified the six-pointed pattern as clearly different from TR+ they preferred it against TR– with a rather high choice frequency (test no. 5). Even more interesting are the tests with a ring. Both ring and six-pointed pattern were discriminated from TR+ with similar choice frequencies (tests nos. 1, 2). However, in a direct comparison between ring and the six-pointed pattern the latter was preferred to 75% (Fig. 8A, test no. 3). That is, the bees accepted the six-pointed pattern as a 'substitute' for TR+ in this comparison, which again indicates a generalization. Even the ring was preferred against TR– (test no. 6 in Fig. 8A). Interestingly, the six-pointed pattern was chosen with the same frequency as the rotated version of TR+ (test no. 4 in Fig. 8A), indicating an asymmetric effect: addition of the perfectly matching TR+ to the inverted TR+ did not make the resulting pattern more attractive, although the degree of overlap was 0 in case of the inverted TR+!

The patterns shown in Fig. 8B allow a first tentative inference about the influence of a straight versus an

interrupted contour (note that the relatively low significance levels in these tests are mainly due to a smaller sample size of only three to four individuals per test). The addition of black in the centre (compared with TR+) led to a high discrimination of the windmill-like pattern from TR+ (test no. 1 in Fig. 8B). However, compared with the large black triangle the windmill pattern was significantly preferred. Thus, either the absence of three black sectors or the interruption of the straight contours allowed this pattern to be discriminated rather well from the black triangle.

4. Discussion

The aim of these experiments was to find evidence in favor or against the hypothesis that foraging bees adhere to a retinotopic-template matching for pattern recognition and discrimination.

4.1. Possible influence of the unrewarded pattern

In the three different training paradigms used here the bees had different optional cues by which they could discriminate the training and test patterns. We used a differential conditioning procedure in which the bee was offered a choice between a rewarded and an unrewarded pattern. This type of training has been found to exert an influence on whether bees rely only on parts of a pattern or on the whole for their classification (cf. Giurfa et al., 1999). For a template matching it may be important whether the outline of TR− (or a test pattern) exceeds the postulated template of TR+ or not (Dill & Heisenberg, 1995; Ronacher & Duft, 1996). Note that in training A and B the unrewarded pattern (as well as most test patterns) did not exceed the outline of TR+, while in training C the TR− covered a black area three times as large as that of TR+. The two unrewarded patterns used in training A and B differed only by the distribution but not by the amount of black areas: for both paradigms the total black area of TR− was one third of that of TR+.

The striking similarity of the results obtained in training paradigm A and B (Figs. 2 and 3) suggests that the bees used mainly features of the TR+ pattern for the recognition and classification of test patterns — which makes sense since a bee's main incentive is to find food sources efficiently. In addition, neither in training paradigms A and B nor in paradigm C was there any indication that the bees had acquired a specific avoidance of features of TR− (see Fig. 5a; cf. however Ronacher, 1992; Giurfa et al., 1999). For this reason in the following we focus on TR+ when investigating a possible retinotopic-template matching process.

4.2. Spontaneous preference for a large black pattern

Comparing the results of training A and C one could argue that the bees had a strong spontaneous preference for large black shapes (i.e. TR+ in training A), which might have biased the preferences acquired during training in some tests (see e.g. Figs. 3 and 5). We checked for this possibility by comparing the first visits and the learning curves obtained in the reciprocal training paradigms A and C, but found no evidence for such a spontaneous preference. (i) The mean learning curves for training A and C were rather similar (indeed the mean choice frequencies were a little bit higher for training C, contrary to the above expectation). (ii) We evaluated the very first approaches and landings performed by each bee on its first voluntary visit to the apparatus (before that visit, the bees were captured at the outdoor feeder and released on the rewarded training stimulus between two and five times). Comparing training A and C, the distributions of first approaches were not significantly different from 50% and from each other ($\chi^2 = 1.63$, n.s.; comparison based on $N = 21$ bees for training A and 47 bees for training B). In both training paradigms the bees showed a preference for the respective TR+ stimulus already in their first voluntary visit (obviously due to the few rewards received while transported passively by the experimenter). In training A, 14 bees directed their first choice to TR+, seven to TR− (n.s. different from 50% according to χ^2 -test), while in training C the first landings of 35 bees were directed to TR+ and of 12 bees to TR− ($P < 0.001$ against 50%). The two distributions of first choices, relative to the respective TR+ stimulus, are not significantly different ($\chi^2 = 0.44$). In case of a strong spontaneous preference for a large black pattern, however, the first choices in training C should have been directed to TR−!

4.3. How to match actual pictures with a stored template

How the bees should find the point in space from which they invoke this postulated template matching process is not well understood, but for the sake of argument we assumed that bees can solve this problem (see e.g. Lehrer, 1993; Zeil, 1993; Wittmann, 1995; Collett & Rees, 1997). There are reports that a template formation could be possible or favored only in case that the bees are allowed to inspect patterns from a close distance, while an experimentally enforced decision from a larger distance would preclude the formation of a template, and prompt the animals to a feature extraction (Srinivasan et al., 1994; Giger & Srinivasan, 1995; Horridge, 1996; cf. also Lehrer, 1993). However, since in our experiments the bees were not restrained in their approach towards the patterns, the experimental design

should not have imposed any such bias towards a feature extraction mechanism.

Different ‘similarity functions’ are conceivable to describe how bees might determine the degree of overlap between retinal patterns and a template (Dill, 1995; Dill & Heisenberg, 1995; Ronacher & Duft, 1996). In earlier experiments the ‘residue’ (*R*), i.e. that part of a test pattern that extends beyond the area occupied by the template, turned out to be important (Dill & Heisenberg, 1995; Ronacher & Duft, 1996). Note that at least in training A and B there was no such residue present in TR –, nor in most of the test patterns (Figs. 2 and 3), and therefore the overlap is the parameter of interest for assessing the similarities of test patterns like those in Fig. 4 (for a detailed account see Ronacher & Duft, 1996). The poor correlation between the choice frequencies and the overlap differences (Fig. 4A) could not be mended by taking into account a distorted projection of patterns upon the compound eye. Two weighting functions that favor the central parts of the patterns led to only a small improvement of the respective correlations between choice frequencies and overlap areas (Fig. 4B and C).

4.4. Several results cannot be explained by a retinotopic-template matching

What kind of experimental evidence enables us to exclude one or the other of the processing mechanisms mentioned in the Section 1? A strong argument against the template hypothesis would be a preference for that pattern of a pair that has the smaller overlap with the supposed template of TR +. Another type of argument, which supports a feature extraction, is generalization (cf. van Hateren et al., 1990; Heisenberg, 1995; Ronacher & Duft, 1996). Both types of evidence indeed have been found in the present account.

In training A, bees discriminated very well between two patterns which — having approximately the same overlap with TR + — should not be distinguished according to the template hypothesis (Fig. 3). Additional evidence against a retinotopic-template matching stems from training C. In several test combinations the bees even preferred a pattern without any overlap to TR + against the alternative pattern that overlapped with TR + (Fig. 5C, Fig. 6B and C). These preferences cannot be reconciled with a template matching by a differential weighting of parts of the patterns. The classifications of patterns depicted in Figs. 6 and 8 indicate a generalization capability of the animals, which is also at odds with the template hypothesis. The bees preferred the rotated TR + against the open triangle and TR – (Fig. 6B), although they were able to discriminate it from TR + in its original position (Fig. 6A, test no. 5). In addition, the bees’ classifications of the ring, the six-pointed pattern, and the windmill

patterns (Fig. 8) also clearly demonstrate a generalization capability.

Taken together, these results provide evidence against the hypothesis that the bees adhered exclusively to a template matching rule in these experiments. Obviously, the bees must have used additional cues for pattern discrimination than evaluating overlap areas. What could these cues have been? Although this question was not in the centre of the present study, our results may give some clues.

4.5. Bees use several cues for their classification of patterns

Somewhat surprisingly, in training paradigm A and B the bees showed no preference for the triangular shape of TR +. Features as e.g. the presence of straight contours in certain orientations or of acute points at certain positions (cf. Anderson, 1977b) had seemingly little value for identifying the positive pattern, there was only a small preference, if any, for the triangle when compared with a large or small disc, and the discs were clearly preferred against the small star or the open triangles (Fig. 2).

For training A and B, most of the results can be explained by the assumption that a sufficiently broad black central area was the main criterion for the bees’ classification of patterns, although in training B the peripheral parts of the patterns offered the most salient cues for discriminating between TR + and TR –. Tentatively one could term this feature ‘compactness’ (cf. Hertz, 1929, 1933, 1934; Zerrahn, 1934; Anderson, 1977a,b). At present, it seems more a matter of semantics whether one includes the feature ‘amount of black area’ into this feature ‘compactness’ or whether one assumes two separate features.

Taken together, the results obtained in training A and B suggest that the bees accepted any pattern as positive that resembled the rewarded pattern in the region around the central reward tube (this is in contrast to Horridge’s 1996 results, who reports that the peripheral parts of the patterns he used were more important for discrimination than their centres). The following observations obtained in training C, however, demonstrate that this simple ‘rule’ derived from the first two training paradigms does by no means exhaustively describe the bees’ classification scheme. (i) A white area within the centre of a pattern cannot be the sole, decisive cue for classification. The very good discrimination between TR + and the open triangles (Fig. 6A, tests nos. 4, 5) demonstrates that in this training the bees must have learnt additional features of TR +. This conclusion is further corroborated by the classifications of ring and six-pointed pattern depicted in Fig. 8A (tests nos. 1–3). Such features may have been e.g. the presence of small black areas, their separation, or

the interrupted contour, leaving an 'open access' to the centre (cf. Fig. 8B). However, further experiments are needed to specify how many and which features were actually used by the bees in this training paradigm (cf. Wehner, 1981; van Hateren et al., 1990; O'Carroll, 1993; Srinivasan et al., 1994; Yang & Maddess, 1997). (ii) Were the bees — having participated in training C — unable to discriminate between so different patterns as depicted in Fig. 5 (tests nos. 1–5)? When interpreting these data one has to consider that a choice frequency near 50% does not necessarily indicate that two test patterns are indiscriminable. Choice frequencies around 50% can also result from a 'similarity trading', i.e. if for the bee's perceptual system the two patterns appear as equally different from TR + (Ronacher, 1979, 1998). Indeed, after training C we observed very high discrimination levels for tests of open triangles or stars against TR + (see Fig. 6A, tests nos. 2–5). This renders a similarity trading the most likely explanation for the choice frequency values around 50% depicted in Fig. 5.

To summarize, the results presented in this study strongly suggest that the bees may perform more sophisticated types of processing than a 'simple' comparison of the amount of overlap between a test pattern and a supposed retinotopically stored template of TR +. It should be emphasized, though, that it is by no means clear whether, from the viewpoint of a nervous system, the evaluation of overlap areas is indeed a simpler process than the extraction of some characteristic features.

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