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Visual scanning behaviour in honeybees

Miriam Lehrer, Rüdiger Wehner, and Mandyam Srinivasan
Department of Zoology, University of Zürich, Winterthurerstraße 190, CH-8057 Zürich, Switzerland

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Summary. 1. Freely flying bees were rewarded with sugar solution on a variety of black-and-white shapes as well as on coloured gratings in various training situations (Table 1). In subsequent dual-choice tests, the bees' discrimination between the various shapes was measured. In addition, the bees were video-filmed while flying in front of the shapes. The scanning patterns thus obtained were then quantified in order to (i) characterize scanning behaviour and its relationship to the geometrical parameters of the scanned shapes, (ii) investigate whether scanning plays a role in pattern discrimination and (iii) examine the influence of training on the characteristics of scanning.

2. The scanning patterns clearly mirror the contours of the scanned shape in all cases (Fig. 3), i.e. the bees fly along the contours contained in the shape. This behaviour does not depend on whether the scanned shape is one that was previously rewarded, or one that is completely novel to the bees.

3. Comparison of the results of quantifying the scanning patterns with the results of dual-choice tests (Fig. 5) reveals that scanning behaviour is independent of discrimination performance.

4. On the average, horizontal scanning directions occur more often than vertical directions (Fig. 4).

5. Variations of the training situation produce measurable differences in scanning behaviour (Fig. 4). However, except in the case of vertical scanning on a vertical grating (Fig. 4), these differences are quite small, indicating that following contours is a largely stereotyped behaviour.

6. Horizontal gratings are very well discriminated from vertical ones even if they offer contrast to only one receptor type, i.e. blue or green (Fig. 8), demonstrating that the direction of contours is visible to the pattern recognition system even under these conditions. However, vertical and horizontal coloured gratings offering only blue-contrast do not elicit contour-following (Fig. 9c and d), whereas gratings offering only green-contrast do (Fig. 9a and b). Thus, the bees' scanning behaviour is colour-blind and most probably governed by the green receptors.

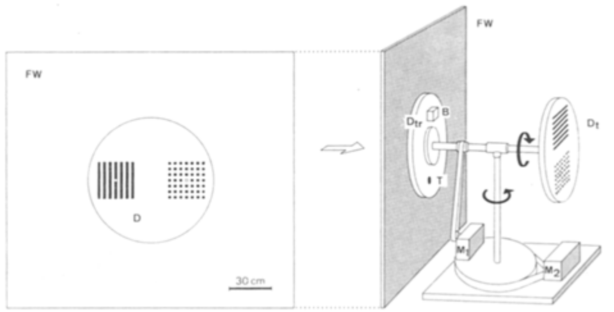
7. We suggest that contour-following is the by-product of a behavioural mode which serves to prevent retinal image movement during flight in front of a contoured visual pattern.

Introduction

When bees are trained to discriminate between two visual patterns displayed on a vertical screen they alternately fixate either pattern by hovering in front of the landing site, i.e. the entrance hole positioned in the geometrical centre of the pattern and leading to the reward (Wehner 1972, 1975). While hovering in front of the entrance hole, the bees maintain a constant flight position with respect to all 6 degrees of locomotor freedom (Wehner and Flatt 1977). During these fixation periods, the bees compare the current image of any novel pattern with an internal (memorized) image of the trained pattern. The better the spatial match between the current image and the memorized image, the more likely the bees are to land on the entrance hole in the centre of the novel pattern (Wehner 1972).

Prior to fixating, however, the bee must locate the fixation point, i.e. the entrance hole. This task is relatively simple when the pattern is, for instance, a uniformly coloured disk (as often used in colour discrimination tasks). The entrance hole is then very conspicuous and is usually located by the bees immediately. However, when the pattern contains contours, as in a linear or radial grating, the entrance hole cannot readily be discerned. In these cases, the bees spend a considerable amount of time flying around slowly near the surface of the pattern, occupying themselves with what might be called scanning the shape. This behaviour – which we originally observed while investigating shape discrimination in bees – certainly deserves to be the subject of a detailed investigation in its own right. How do bees scan shapes, under what circumstances and why?

In the present study, we first video-film flight paths performed in front of a variety of black-and-white shapes, after various training procedures as well as in different test situations. Next, we ask if there is a relationship between the bees' scanning behaviour and their performance in discriminating shapes. Finally, by presenting the bees with shapes which contain specific colour contrasts, we examine the role that colour-contrast plays in scanning behaviour. We conclude by discussing the properties and the possible function of the bees' scanning behaviour.



a b

Fig. 1a, b. Apparatus. **a** Frontal view as encountered by the freely flying bees. The vertical disc *D* carrying the shapes fits into a round window cut in the front wall. The two shapes shown on *D* are one example of the various combinations and modes of presentation used in the course of the experiments. **b** Side view. *FW* Front wall. The bees approach the front wall from the direction marked by the *arrow*. D_{tr} Disc used during training; D_t Disc used during testing; *B* Dark box containing feeder with sugar solution; *T* Plexiglass tube penetrating the centre of the shape. All tubes are closed by means of black plugs except for the one leading to the reward. *M1* Motor which rotates the disc by remote control. Box *B* is weighted and mounted on a ball bearing, so that it remains vertically oriented when the disc rotates. *M2* Motor which rotates, by remote control, the whole pattern-carrying device, so that the training disc can be replaced by the testing disc and vice versa

Materials and methods

Individually marked freely flying bees (*Apis mellifera*) were trained to visit the experimental apparatus (Fig. 1) where two visual patterns (shapes) were simultaneously offered to them on a vertical disc. Each shape was mounted on a transparent plexiglass tube inserted through its centre. One of the shapes was rewarded, i.e. the tube led to a small box containing a feeder which offered sucrose solution. The alternative shape carried no reward: The tube at this shape was closed. The rewarded and the unrewarded shapes were interchanged every 2–3 rewards by turning the disc through 180°, in order to prevent the bees from using positional cues in the task of recognizing the rewarded shape. Turning was done by means of a remote-controlled electrical motor (M1 in Fig. 1).

In a first series of experiments, black-and-white shapes (Fig. 2) of high contrast (0.83) were used pairwise. (For calculations of contrast, see Srinivasan and Lehrer 1984). Each pair of shapes served in two different sets of training procedures, in which 3–5 new bees were involved each time. In one set of experiments, the two shapes were presented one beside the other, i.e. their centres were positioned along a horizontal line (horizontal mode of presentation, see example in Fig. 1a). In a second set of experiments, the same pairs of shapes were presented one above the other (vertical mode of presentation, see example in Fig. 1b).

After an initial period of training comprising about 30 rewarded visits (to ensure that the learning process was complete; see, for example, Fig. 8), the bees were individually tested by presenting them with either the training shapes or other pairs of shapes at the same location but on a separate disc (see Fig. 1), in order to exclude the influence of scent-cues. During tests, neither shape was rewarded. The individual bees were video-filmed for a period of about 2 min while flying in front of one of the two shapes. The video camera (National, WV-1350 AE/G) was focussed on the surface of the shape, to ensure that only a bee flying very near to it was selected for the analysis of flight-paths. (A bee flying further than 3 cm from the surface of the shape was out of focus. This measure ensured that the filmed bee had chosen to search for the reward on the filmed shape.) Between tests, additional 3–5 rewards were offered (on the training disc).

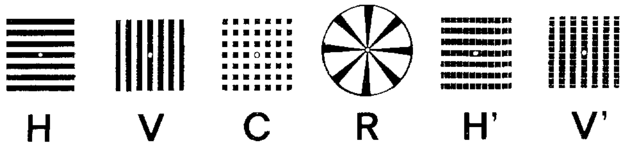


Fig. 2. Black-and-white shapes used in the study. Shapes *H* and *V* Squares (280 × 280 mm^2) containing parallel black and white stripes each 20 mm wide. *H* Horizontal grating, *V* Vertical grating. Shape *C* Checkerboard. The black stripes of shape H (or V) are broken into squares of 20 × 20 mm^2 separated by 20 mm. Shape *R* Radial grating. The disc (∅ = 360 mm) contains eight radial sectors, each 22.5° wide. Shapes *H'* and *V'* Horizontal and vertical gratings as *H* and *V*, but with black stripes broken into 18 mm long rectangles separated by white intervals of 2 mm

Table 1 lists the pairwise combinations in which the shapes shown in Fig. 2 were offered to the bees during various sets of experiments. The shapes rewarded during training (left column) are indicated by bold letters, while the ones that were video-filmed during the various tests (columns I–IV) are shown encircled. The alternative training and test shapes are shown in brackets. In the tests, the mode of presentation was always the same as during the preceding training. In the tests shown in column I, the test situation was identical with the training situation and the filmed shape was the one which had been rewarded during training. In the tests shown in columns II, III and IV, the video-filmed shape was different from that previously rewarded.

Table 1. The pairwise combinations of black-and-white shapes used in this study
Left column: Combinations used during training in experiments 1-6. The rewarded shape is shown in bold letters, the unrewarded alternative shape in brackets. Columns I-IV: Combinations used in dual-choice tests following training and during video-filming. The recordings on the shapes shown encircled were analysed to obtain scanning patterns. For horizontal and vertical mode of presentation, see text

Exp.	Training	Tests				Mode of presentation
		I	II	III	IV	
1	**V** (*V'*)	Ⓥ (*V'*)	Ⓗ (*V*)	Ⓒ (*H*)	Ⓡ (*C*)	Vertical
2	**H** (*H'*)	Ⓗ (*H'*)	Ⓥ (*H*)	Ⓒ (*V*)	Ⓡ (*C*)	Vertical
3	**V** (*V'*)	Ⓥ (*V'*)	Ⓗ (*V*)	Ⓒ (*H*)	Ⓡ (*C*)	Horizontal
4	**H** (*H'*)	Ⓗ (*H'*)	Ⓥ (*H*)	Ⓒ (*V*)	Ⓡ (*C*)	Horizontal
5	**C** (*R*)	Ⓒ (*R*)	Ⓗ (*R*)	Ⓥ (*R*)	Ⓡ (*V*)	Vertical
6	**C** (*R*)	Ⓒ (*R*)	Ⓗ (*R*)	Ⓥ (*R*)	Ⓡ (*H*)	Horizontal

Playing back the video tapes at 1/36 of their original speed (the real-time interval between successive frames being 20 ms), the bee's flight paths were transferred onto transparent cellophane sheets by following the bee with a fine ink pen directly on the monitor. Each flight path thus obtained was analyzed by superimposing it upon a grid of 13 × 13 equal squares, each representing an area of 20×20 mm^2 of the original square shapes (H, V, C, Fig. 2) or 24×24 mm^2 of the radial grating (shape R, Fig. 2), respectively. Within each elementary square, four different types of flight-elements were distinguished and counted: straight flight in horizontal (a), vertical (b), and oblique direction (c), as well as curved elements, such as arcs, U-turns and turns on the spot (d). The relative frequency of occurrence of each type (a)–(d) was then calculated for each test.

Horizontal and vertical flight directions were defined by $\alpha = 180° \pm 15°$ and $\alpha = 90° \pm 15°$, respectively, α being the angle between the observed direction of flight and the horizontal.

Finally, the individual flight paths concerning each particular test were superimposed to yield a scanning pattern for each tested shape.

In addition to video-recordings, the bees' relative preferences for the two test shapes (Table 1) were measured in separate dual-choice experiments carried out shortly before each filming. Each test lasted for 4 min, the positions of the two shapes being interchanged after the first 2 min. Two types of behaviour were distinguished and counted: (i) touching the entrance of the tube (T in Fig. 1) with either the antennae or forelegs was scored as 1 point, (ii) landing on the tube was scored as 2 points. Choice frequencies were calculated from the sums of points obtained for each shape.

It can be seen in Table 1 that each of the test shapes H, V, C and R (the horizontal grating, the vertical grating, the checkerboard shape and the radial grating, respectively) was video-filmed six times, each time after a different training procedure (experiments 1–6) and that, in the course of the experiments, each of these four shapes was tested for discrimination against each of the other three on several occasions.

In a second series of experiments, the same horizontal and vertical gratings (shapes H and V, Fig. 2) were used as before, but this time they were made out of coloured rather than black and white papers. Two different colour-combinations, denoted by G and B, were used, each offering a specific colour contrast: Combination G containing contrast only in the green region of the spectrum (green-contrast, 0.40) was chosen so as to stimulate mainly the green receptors, while combination B contained only blue-contrast (0.56), so as to stimulate mainly the blue receptors. Both combinations possessed the same brightness-contrast (0.21). (Colour combinations and contrast calculations are as specified in Srinivasan and Lehrer 1984.)

In these experiments, the mode of presentation was always horizontal. For each combination G and B, four experiments were carried out: 1) A training experiment in which the vertical grating (rewarded) and the horizontal grating (unrewarded) were offered simultaneously. The bees' discrimination between the two training shapes was measured several times at intervals of about 5 rewarded visits. 2) Preliminary video-recordings in which the same bees as in (1) were individually filmed while scanning the previously rewarded (vertical) grating. From the obtained scan paths, the proportions of the various scanning elements were calculated as explained above. Thus, experiments (1) and (2) served to *quantify* the learning performance and the scanning behaviour for each of the two colour combinations. 3) New bees were rewarded 10 times on the horizontal grating and then individually filmed on that shape. The alternative shape in both training and filming was the vertical grating of the same colour-combination (G or B, respectively). 4) New bees were rewarded 10 times on the vertical grating and then individually filmed on this shape, the alternative shape being the horizontal grating. Thus, in experiments (3) and (4), two scanning patterns were obtained for each of the colour-combinations G and B, one on the vertical and one on the horizontal grating.

Results

Black-and-white shapes

1. Scanning patterns and choice frequencies. From just looking at the bees' scanning patterns, it is very easy to classify them into four groups, without even having been told that four different test-shapes had been used. Figure 3 shows eight examples (two for each of the four shapes) out of a total of 24 which were analysed (six for each shape). Each scanning pattern clearly mirrors the contours of the scanned shape.

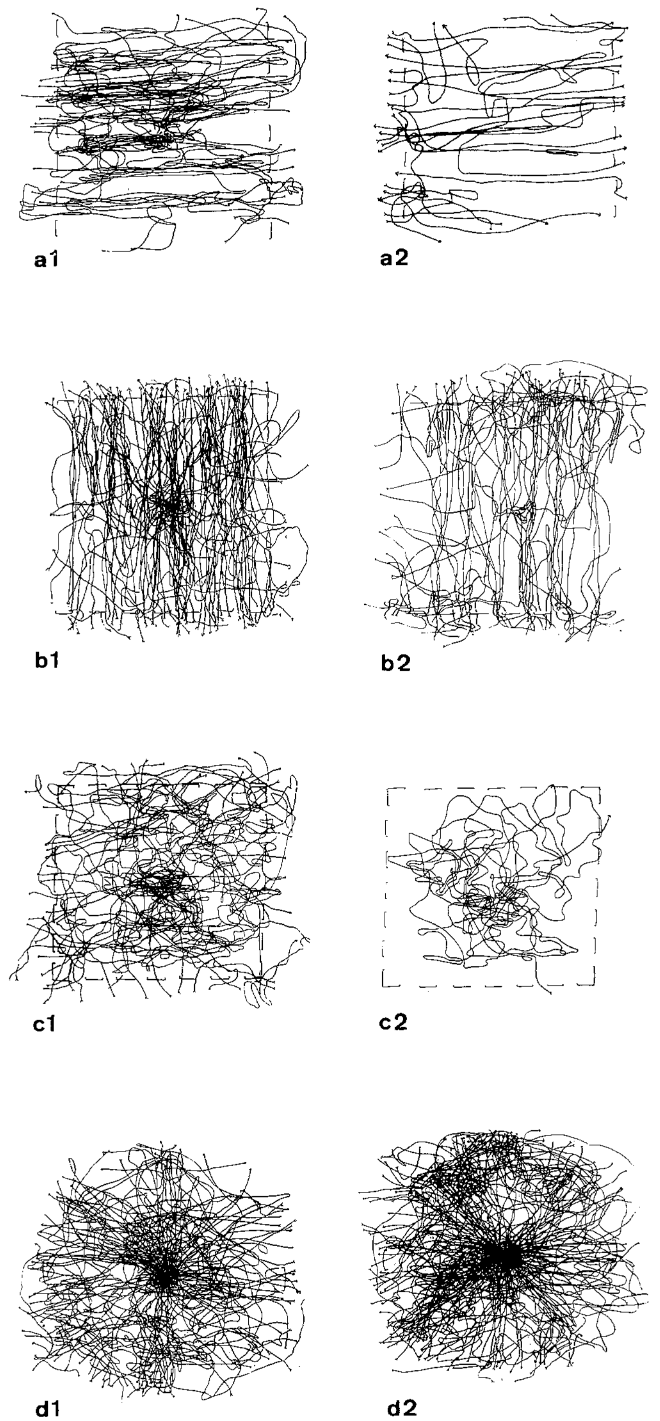


Fig. 3. The bees' scanning behaviour as performed in front of horizontal (**a**) and vertical gratings (**b**), checkerboards (**c**) and radial gratings (**d**). For each shape, two examples out of the total of six video recordings (Table 1) are shown. With the exception of **c2**, which shows a single scanning sequence of an individual bee, scanning sequences of 2–4 bees individually filmed during a total period of about 2 min have been superimposed within each graph. **a** Scanning patterns obtained on the horizontal grating. *1:* Test 4/I. 2 individual bees after 24 rewards on this shape. (All values given for the number of rewards are mean values of the bees involved.) *2:* Test 1/II. 3 individual bees after 41 rewards on the vertical grating. **b** Scanning pattern obtained on the vertical grating. *1:* Test 1/I. 4 individual bees after 35 rewards on this shape. *2:* Test 5/III. 3 individual bees after 28 rewards on the checkerboard shape. **c** Scanning patterns obtained on the checkerboard shape. *1:* Test 4/III. 3 individual bees after 40 rewards on the horizontal grating. *2:* Test 5/I: A single flight sequence of an individual bee after 40 rewards on this shape. (For quantification of this test, other sequences concerning all other bees taking part were used in addition.) **d** Scanning patterns obtained on the radial grating. *1:* Test 1/IV. 3 individual bees after 33 rewards on the vertical grating. *2:* Test 2/IV. 2 individual bees after 36 rewards on the horizontal grating

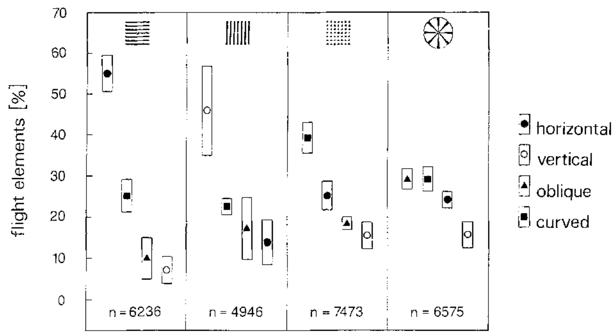


Fig. 4. Distribution of horizontal (●), vertical, (○), oblique (▲) and curved (■) flight elements as calculated from the analyses of the scanning patterns obtained for each of the shapes H, V, C and R (from left to right). Data are based on six scanning patterns (Table 1) obtained for each shape. Height of each box indicates ± standard deviation. n = total number of flight elements

Figure 4 presents the frequencies of the four different types of flight elements obtained on each of the four filmed shapes (see Methods). Each point depicts the mean value and standard deviations as obtained from six recordings per shape (see Table 1). Within each panel, the frequencies (ordinate) of the four types of scanning elements considered are arranged in descending order. Clearly, the bees tend to follow the contours of the shape: The horizontal grating is scanned mainly in the horizontal direction, the vertical grating mainly in the vertical direction. The lowest frequency of horizontal flight elements occurs on the vertical grating, the lowest frequency of vertical elements on the horizontal grating. On the checkerboard shape, curved elements occur significantly more often than on the radial grating ($P < 0.001$, t-test): they mirror the comparatively frequent changes of contour direction present in this shape. On the radial grating, on the other hand, oblique flight is significantly more frequent than on the checkerboard shape ($P < 0.001$, t-test): the radial grating contains oblique contours, while the checkerboard shape does not.

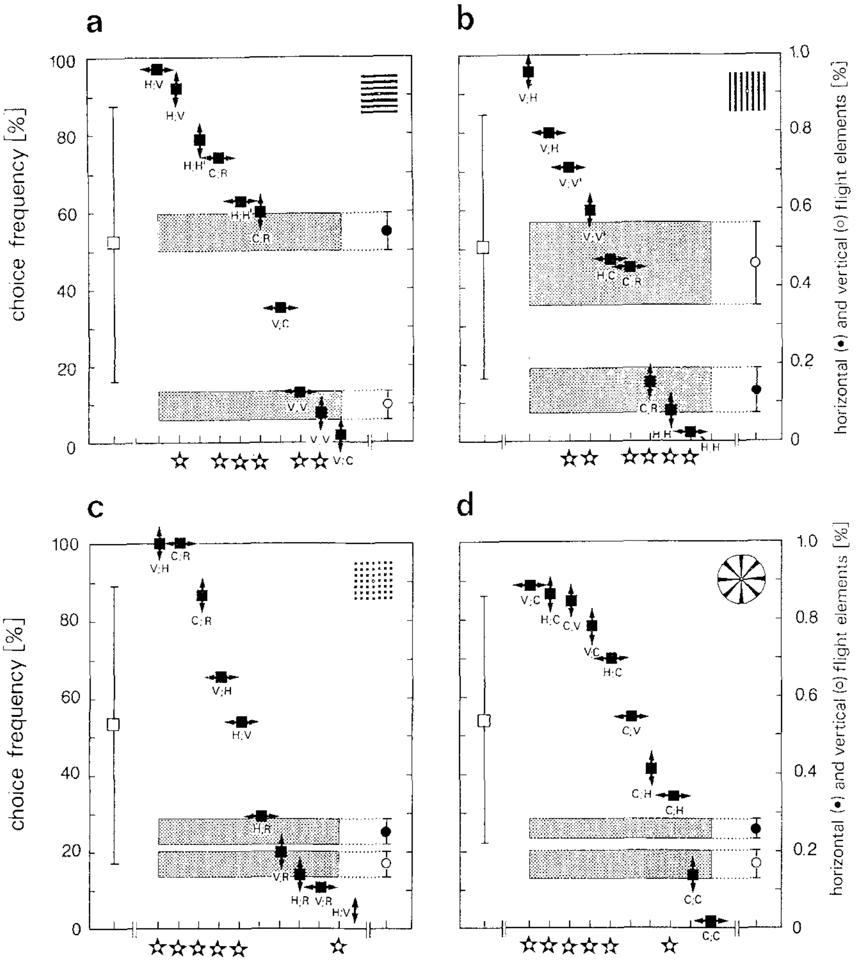


Fig. 5a–d. Choice frequencies (*left ordinate, filled squares*) obtained on the horizontal grating (**a**), on the vertical grating (**b**), on the checkerboard shape (**c**) and on the radial grating (**d**). Horizontal and vertical double-arrows superimposed on the symbols denote horizontal and vertical mode of presentation, respectively. Capital letters under each symbol show the previously rewarded shape (first letter) and the alternative shape used during test (second letter): H = horizontal grating; H' = broken horizontal grating; V = vertical grating; V' = broken vertical grating; C = checkerboard; R = radial grating. Mean values and standard deviations of the choice frequencies in all tests taken together are shown on the left-hand side of each graph (*open square*). Total number of choices n: (**a**) $n=627$; (**b**) $n=686$; (**c**) $n=569$; (**d**) $n=701$. Stars along the abscissa depict the tests in which the regarded shape (inset in each graph) was video-filmed. Filled and empty *circles* (*right ordinate*) depict mean frequencies of horizontal and vertical flight elements, respectively, obtained from the analyses of the scanning patterns. The standard deviations of these values (shaded) are superimposed on the graphs so as to demonstrate the considerable differences between the results of discrimination tests and the frequencies of scanning elements obtained in the very same tests

Figure 5 presents the results of the dual-choice experiments. Each panel shows, for one of the shapes, the choice frequencies that it elicited (filled squares, left ordinate) in all of the tests in which it was used.

Obviously, the choice frequencies in favour of a given shape can take any value between 0 and 100%: dual-choice experiments measure discrimination, and discrimination depends in all cases upon the shape which has been rewarded during training and upon the alternative shapes offered during the test. The rewarded shape and the alternative test shape are therefore indicated in the figure.

In all tests other than those of type I (see Table 1), the filmed shape was totally novel to the bees. Excellent discrimination is obtained when the novel shape is tested against the previously rewarded shape (Experiments 1–4, tests of type II). In all other cases, both test shapes were different from the rewarded training shape. In these tests, the bees clearly prefer the shape the geometry of which has more in common with that of the rewarded shape.

For the sake of comparison, the mean frequencies (±SD) of the horizontal and vertical flight elements obtained from the video-filmings are also included in Fig. 5 (right ordinate and bars superimposed on each graph): Clearly, the scanning patterns elicited by a given shape are essentially independent of whether or not that shape was rewarded during training and of how well it is discriminated from other test shapes. The proportions of the various scanning elements (horizontal, vertical, oblique and curved) are very similar when the shapes are identical, but very different when the shapes are different (see also Fig. 4).

Obviously, there is no correlation between the measured choice frequencies and the measured proportions of the various scanning elements. Even when the frequencies of flight elements showed some variability, as in the case of the vertical scanning elements on the vertical grating (Fig. 5b), there is no correlation between the frequencies of the vertical flight elements and the choice frequencies obtained in the very same tests ($0.3<P<0.4$, linear regression analysis). In all other cases, the lack of correlation is so obvious that statistical examination is superfluous.

2. Spontaneous preferences. Comparing the frequencies of horizontal and vertical flight elements in Fig. 4a and b, the average frequency of horizontal scanning on the horizontal grating is higher than the average frequency of vertical scanning on the vertical grating (54.6% ± 4.6% versus 45.9% ± 11.4%), and the average frequency of horizontal flight elements on the vertical grating is higher than that of vertical flight elements on the horizontal grating (13.0% ± 5.8% versus 8.1% ± 3.6%). This finding suggests that the bees prefer to scan horizontally rather than vertically. However, the differences do not differ statistically ($0.1 < P < 0.2$ in each of the above comparisons, t-test), showing that contour-following is the primary factor responsible for the scanning patterns obtained on these shapes. The preference for the horizontal flight direction is therefore much more pronounced in front of the checkerboard shape (Fig. 4c) and the radial grating (Fig. 4d), which offer horizontal and vertical contours in exactly equal amounts. Here, horizontal flight elements are significantly more frequent than vertical flight elements: 25.1% ± 3.8% versus 16.3% ± 3.5% ($P < 0.02$) in the case of the checkerboard shape, and 24.6% ± 2.1% versus 16.5 ± 3.7% ($P < 0.01$) in the case of the radial grating (t-test).

In fact, the bees' preference to fly horizontally can lead to difficulties during training. When the mode of presentation is horizontal and the training shapes are interchanged for the first time (see Methods), the bees find the rewarded shape at its new location within less than 1 min. If the mode of presentation is vertical, some bees take up to 16 min to locate it, and some never find it.

The bias toward scanning in the horizontal direction may reflect the bees' natural tendency to fly in the horizontal plane while foraging.

The bees' preference for the horizontal flight direction may be responsible for the comparatively high standard deviation obtained for the vertical scanning on the vertical grating (Fig. 4b. See also Sect. (3)).

3. The influence of the training procedure on the scanning patterns. The details of the bees' scanning pattern in front of a given shape show small variations among the different tests (Fig. 4). This variability could be due to the differences in the training procedure, namely, to (i) the shape that is being rewarded during training and (ii) the mode of presentation. The rewarded shape, because it is scanned in the direction of its contours previous to each reward, may cause the bees to learn, at least to some extent, to fly preferentially in that direction even when presented with a different shape. The mode of presentation, on the other hand, determines the direction in which the bees must fly from one shape to the other, and could thus increase the probability of scanning in this direction. To test these two possibilities, we compare scanning patterns obtained (i) after rewarding different shapes in a constant mode of presentation, and (ii) after rewarding a particular shape in different modes of presentation.

In Fig. 6, the frequencies of horizontal and vertical flight elements (filled and open circles, respectively) on each of the filmed shapes are arranged according to the training procedure applied prior to testing. The rewarding training shape is depicted below the abscissa. Tests carried out using the horizontal mode of presentation are shown on the left-hand side, and those carried out using the vertical mode of presentation on the right-hand side of each graph (a–d).

In the horizontal mode of presentation, horizontal scanning elements (filled circles in Fig. 6) are always more frequent when the horizontal grating is rewarded than when the vertical grating is rewarded[1]. Vertical scanning elements (open circles), on the other hand, are always more frequent when the vertical grating is rewarded[2].

In the vertical mode of presentation, the frequencies of vertical scanning elements (open circles) are in two of the four cases higher after rewarding the vertical grating than after rewarding the horizontal one[3]. In the remaining two cases, no differences can be found between the frequencies of vertical scanning elements[4]. The frequencies of horizontal scanning elements (filled circles) in the vertical mode of presentation, however, are higher after training on the horizontal grating than after training on the vertical grating in only one case[5]. In the other three cases they are lower[6].

In conclusion, the frequencies of horizontal and vertical scanning elements depend somewhat upon the rewarded training shape in eleven out of sixteen

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[1] Compare 4/I with 3/II in Fig. 6a, 4/II with 3/I in Fig. 6b, 4/III with 3/III in Fig. 6c and 4/IV with 3/IV in Fig. 6d

[2] Compare 3/II with 4/I in Fig. 6a, 3/I with 4/II in Fig. 6b, 3/III with 4/III in Fig. 6c and 3/IV with 4/IV in Fig. 6d

[3] Compare 1/II with 2/I in Fig. 6a and 1/I and with 2/II in Fig. 6b

[4] Compare 1/III with 2/III in Fig. 6c and 1/IV with 2/IV in Fig. 6d

[5] Compare 4/II with 3/I in Fig. 6b

[6] Compare 2/I with 1/II in Fig. 6a, 2/III with 1/III in Fig. 6c and 2/IV with 1/IV in Fig. 6d

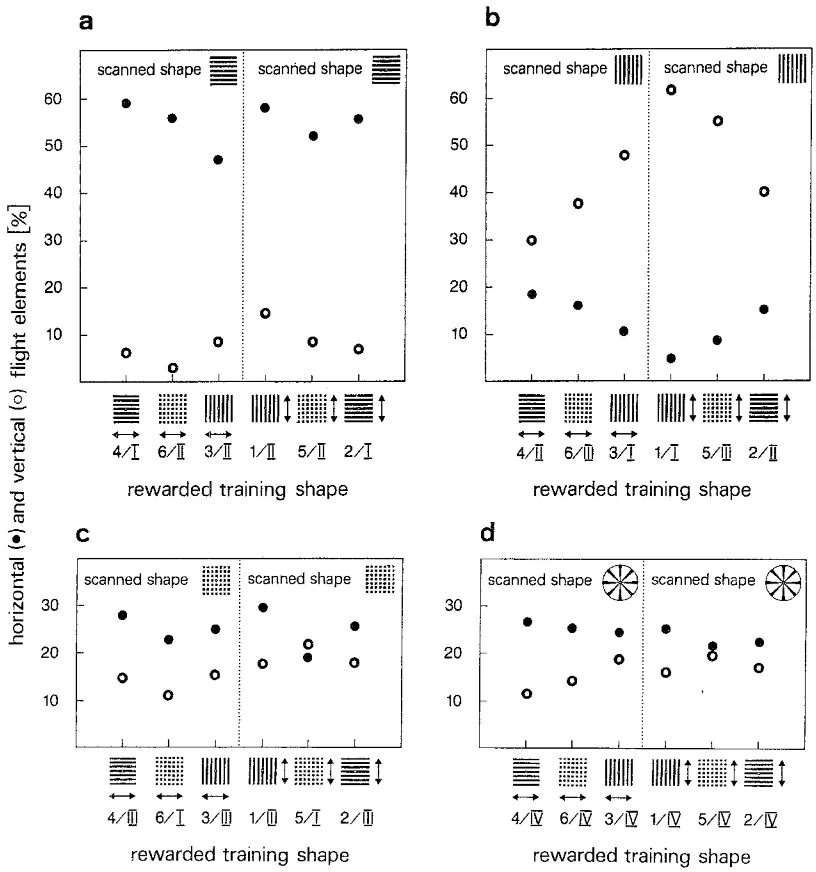


Fig. 6a–d. Influence of training procedure on the bees' scanning behaviour. *Ordinate:* Frequency of horizontal and vertical scanning elements (*filled* and *open circles,* respectively) obtained on the horizontal grating (**a**), on the vertical grating (**b**), on the checkerboard shape (**c**) and on the radial grating (**d**). Data were obtained from six recordings on each shape. The rewarded shape is depicted below the abscissa. Horizontal and vertical *double-arrows* denote horizontal and vertical mode of presentation, respectively. In each graph, the three tests shown on the left- and right-hand side were performed in the horizontal or vertical mode of presentation, respectively

comparisons. This dependence is more pronounced in the horizontal than in the vertical mode of presentation. The effect is strongest when vertical scanning is performed on vertical gratings (Fig. 6b, open circles).

The influence of the mode of presentation is observed best in those cases in which the rewarded training shape is not expected to influence the scanning direction, i.e. after training on the checkerboard shape. Each of the four filmed shapes was offered to the bees after such a training in both the horizontal and the vertical mode of presentation. In these cases, the frequencies of horizontal and vertical scanning elements, respectively, are always higher when the regarded scanning direction coincides with the direction of the mode of presentation than when it is perpendicular to it[7].

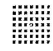
It is also here that vertical scanning on the vertical grating is affected most of all (Fig. 6b, open circles).

[7] For horizontal scanning direction (filled circles in Fig. 6), compare 6/II with 5/II in Fig. 6a, 6/III with 5/III in Fig. 6b, 6/I with 5/I in Fig. 6c and 6/IV with 5/IV in Fig. 6d; for vertical scanning directions (open circles), compare 5/II with 6/II in Fig. 6a, 5/III with 6/III in Fig. 6b, 5/I with 6/I in Fig. 6c and 5/IV with 6/IV in Fig. 6d

The influence of the mode of presentation on the scanning behaviour could be a simple consequence of the fact that the bees often enter the shape coming straight from the other, and also leave the shape to fly directly to the other. Thus, it is conceivable that the horizontal mode of presentation tends to increase the proportion of horizontal scanning elements and the vertical mode of presentation the proportion of vertical scanning elements, because, at least in the initial and final phase of the scanning sequence, the flight direction coincides with the direction of the mode of presentation. One would expect this effect to be particularly noticeable in those cases in which entries to and exits from the shape occur very frequently, i.e. in the cases in which the speed of scanning is high and the duration of individual scanning sequences short.

Table 2 shows the average length of individual scanning sequences, defined as the number n of flight elements divided by the number E of entries into the shape, for each of the four filmed shapes in all six tests in which it has been filmed. The shortest scanning sequences occur on the horizontal and vertical gratings: while flying along the contours of the grating, the bees are swept out

Table 2. Mean length of single scanning sequences as expressed by the coefficient n/E (n = number of scanning elements; E number of entries to the shape)

Scanned shape				
Average length of scanning sequences (n/E)	23.7	21.0	28.5	50.3
Number of scanning elements (n)	6,236	4,946	6,575	7,473
Number of entries (E)	263	236	231	149

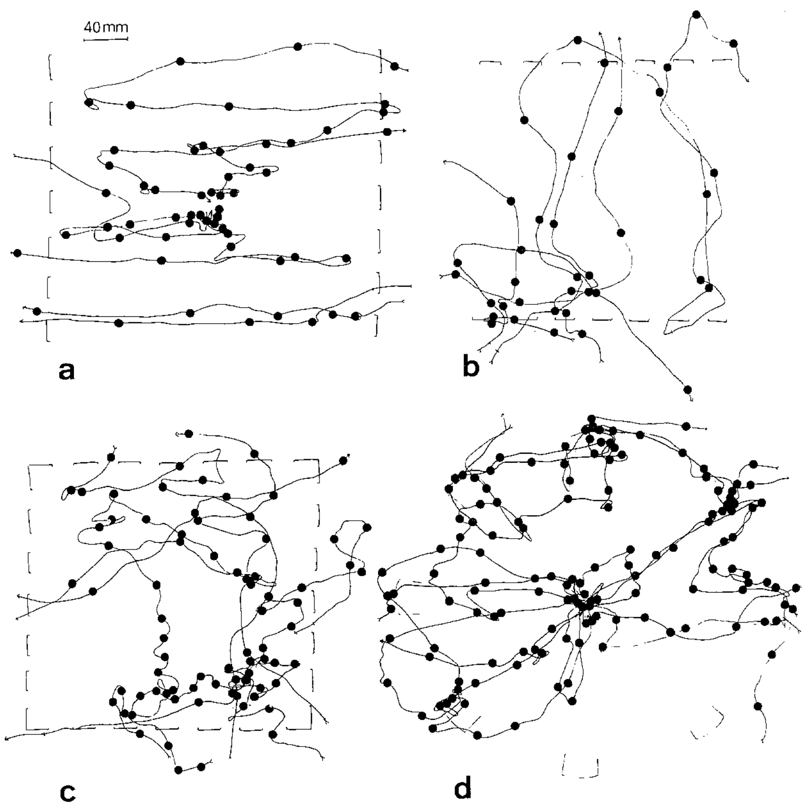
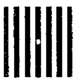
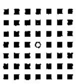
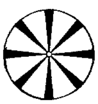


Fig. 7a–d. Speed of scanning examined in frame-by-frame analyses of the video recordings. *Filled circles* along the flight trajectories depict the position of the bee's head every 167 ms. The examples shown for each of the four filmed shapes (**a–d**) were randomly chosen from various tests: scanning on the horizontal (**a**) and vertical grating (**b**), the checkerboard shape (**c**) and the radial grating (**d**)

of the shape every now and then (see Fig. 3a and b), and, due to the small proportion of curves and turns performed on these shapes, the average speed of flight is high (Fig. 7a and b). On the checkerboard shape, on the other hand, the average speed of flight is lower due to the large proportion of curved elements (Fig. 7c). But this shape does more than that: As there are no contours extending directly out to the boundaries, the bees are trapped on this shape and scan for very long periods of time (Table 2). Some sequences could have lasted longer than 2 min, had the test been prolonged. One example of a very long scanning sequence on the checkerboard shape is shown in Fig. 3c2. Such was never observed on any of the other shapes. The radial grating occupies an intermediate position with regard to both speed (Fig. 7d) and length of individual scanning sequences (Table 2). There is a straight path out of this shape, but the contours often take the bee to the centre of the shape, where it usually lands and from where it usually continues scanning in a new direction.

Thus, if the mode of presentation were to play any role in the bees' scanning behaviour, one would expect it to influence mainly the scanning of the horizontal and vertical gratings. However, the results of statistical examinations (Table 3) show that this is not the case. Contrary to the above prediction, horizontal scanning elements in the horizontal mode of presentation are significantly more frequent than in the vertical mode of presentation not on the horizontal or vertical grating, but rather on the radial grating. Vertical scanning elements, on the other hand, are significantly more frequent in the vertical mode of presentation than in the horizontal mode of presentation not

Table 3. Statistical comparison (t-test) of the average frequencies of horizontal and vertical scanning elements obtained on each shape in the horizontal mode of presentation (horizontal double-arrow) versus those obtained in the vertical mode of presentation (vertical double-arrow)

Scanned shape	Horizontal scanning elements		Vertical scanning elements	
	↔	↕	↔	↕
	54.5±6.2	54.9±3.8	6.0±2.2	10.2± 3.6
	$0.8<P<0.9$		$0.05<P<0.1$	
	16.0±4.5	10.0±6.1	39.1±8.4	52.7±10.8
	$0.1<P<0.2$		$P<0.02$	
	25.1±2.7	25.2±5.4	13.6±3.0	19.1± 1.3
	$0.9<P<0.95$		$P<0.001$	
	26.0±1.0	23.2±2.0	14.5±4.0	18.5± 2.4
	$P<0.05$		$0.1<P<0.2$	

Same data as in Fig. 6, but comparing pooled data in the left half of each panel with pooled data in the right half

only on the vertical grating (a result which is in accord with the expectation), but also on the checkerboard shape (a result which is not).

Thus, flying in a particular direction, i.e. in the direction of contours contained in the rewarded shape or in the direction of mode of presentation, is not incorporated into the learning process. In other words, the scanning performed on the rewarded shape during training is not reproduced on a shape which offers contours oriented in a different direction, although scanning on a novel shape can be slightly influenced by the previously applied training procedure.

The finding that it is mainly vertical scanning on the vertical gratings that can be strongly enhanced by the training procedure (Fig. 6b) suggests that training on the vertical grating and/or in the vertical mode of presentation might act to suppress the bees' spontaneous preference to fly

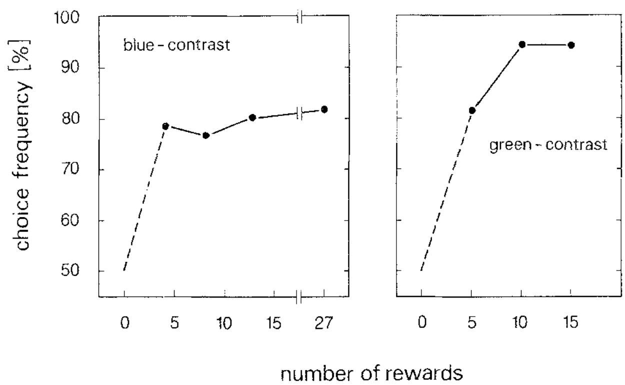


Fig. 8. Discrimination between horizontal and vertical gratings displayed in blue-contrast (*left*) and green-contrast (*right*). In both cases, the vertical grating was rewarded during training. *Abscissa:* average number of rewarded visits. *Ordinate:* Choice frequencies in favour of the vertical grating

in the horizontal direction (see Sect. 2). In the scanning patterns performed in front of the other shapes (Fig. 6a, c, d), this preference is superimposed upon the influence of the training procedure.

Colour-contrast gratings

For each of the two colour combinations – blue-contrast (B) and green-contrast (G) – the horizontal and the vertical gratings used possessed the same dimensions and spatial frequencies as the black-and-white gratings used before (shapes H and V, Fig. 2). First, the bees' performance in discriminating between the vertical and the horizontal grating was measured for each colour combination. The bees rapidly learn to discriminate well between the vertical and the horizontal grating (CF>80%) (Fig. 8). Obviously, the direction of the contours is visible to the bees in both cases, and therefore the colour-contrast contained in the gratings (green-contrast or blue-contrast) must be perceived well by the bees.

Figure 9a and b shows the scanning patterns obtained for the colour-combination G on the horizontal (a) and on the vertical grating (b), as do Fig. 9c and d for the colour-combination B. In comparing Fig. 9a and b with c and d one realizes that green-contrast elicits flying along contours, while blue-contrast does not. The same conclusion can be drawn from the frequencies of the scanning elements obtained from the preliminary recordings on the vertical gratings B and G (see Methods): The proportion of vertical scanning elements on the grating B (blue-contrast) is 11.8%, while it is 44.0% on the grating G (green-contrast) (see also Fig. 4b, where the black-and-white shapes offered equally high contrasts (0.83) to both the blue and the green receptors).

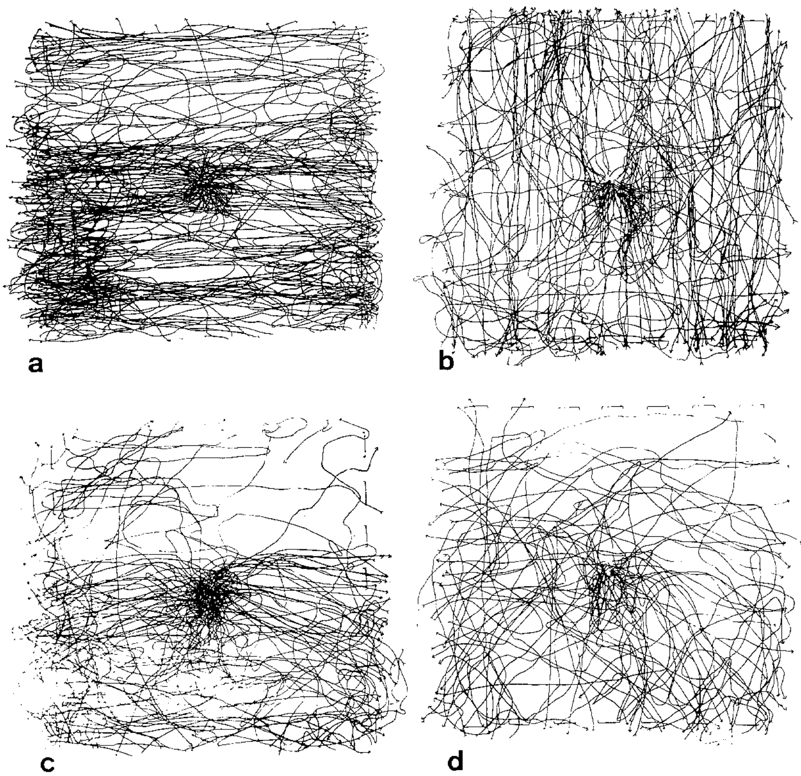


Fig. 9 a–d. Scanning patterns of bees flying in front of horizontal (**a, c**) and vertical gratings (**b, d**). **a, b** green-contrast gratings. **c, d** blue-contrast gratings

Summing up, the contour-following behaviour has two important properties: it is (i) largely independent of the training or test situation, and (ii) mainly governed by the green-channel and thus, most likely, colour-blind.

Discussion

Scanning and visual discrimination

Active movements of the eyes (or the head or the whole body) relative to a stationary visual object have often been described, in humans as well as in many arthropods (see Wehner 1981, pp. 443–451). The term scanning, however, has usually been applied only to those situations in which this behaviour is used in pattern recognition. Thus, eye movements in humans are termed scanning by Noton and Stark (1971), who argue that this behaviour might play a role in the learning and subsequent recognition of patterns. On the other hand, Yarbus (1967) has shown that eye movements are not at all necessary in performing this task. Anderson (1979), who recorded flight paths of bees that had been trained to an unstructured black bar, observed that when the bees are presented with a bar that contains novel areas (such as subdivided contours, additional contours or areas of high contour density), they spend an increased amount of time exploring the novel area. As Anderson found that after the inspection the bees fly away rather than land on the shape, he conjectured that this behaviour might be involved in the process of pattern recognition.

There is, however, strong evidence, that the bees' scanning behaviour does not serve pattern discrimination: (i) In dual-choice experiments bees are found to recognize the correct pattern (i.e., the one previously rewarded) before they even come near enough to scan it. The bees entering the experimental area are observed to aim initially toward one of the two patterns, and, if the patterns differ sufficiently, it is always the rewarded pattern that they approach. (ii) The final decision to select a particular pattern takes place not during the scanning sequences performed in front of the pattern, but rather during a fixation phase in which the bee hovers nearly stationary in front of the pattern (Wehner 1972, 1975; Wehner and Flatt 1977). (iii) Testing the bees' spontaneous preferences to gratings of different stripe width and spacing, Anderson (1977) showed that horizontally oriented gratings (presented in a vertical plane) are more attractive to the bees when their contour density is higher, i.e. when they possess a higher spatial frequency. Anderson does not particularly mention how the bees scanned the shapes he had offered, but presumably they followed contours the same way our bees did. If scanning were involved in evaluating contour density then the bees should cross the contours rather than fly along them. (iv) Our present results show that following contours is a spontaneous behaviour which does not depend on the bees' performance in pattern recognition or pattern discrimination. (v) Visual recognition and scanning behaviour appear to be governed by systems having different kinds of spectral dependence: While pattern recognition certainly is not colour blind (see Fig. 8), contour-following is (see Fig. 9).

Why do bees follow contours?

In view of the above arguments, we use the term scanning in a purely descriptive sense. We have neither postulated nor found that this behaviour is used in the process of distinguishing between the shapes. Why, then, do bees follow contours?

We suggest that contour-following is a by-product of a behavioural mode used in the visual stabilization of flight, e.g. some kind of optomotor response. Numerous earlier studies of optomotor responses have demonstrated that *tethered* animals exhibit compensatory turning responses which tend to reduce motion of the visual image on the retina, indicating that the animal is not able to judge solely on the basis of retinal input whether retinal movement is a consequence of its voluntary locomotor activity or of passive drifting. Thus, during flight in front of the shape, relative motion of the image on the retina in a direction perpendicular to the contours would evoke a counteractive optomotor response, while motion along the contours would not. As a result, flight occurs primarily in the directions parallel to the contours, motion in other directions being inhibited by the optomotor reaction. A similar argument was used to explain why freely walking *Drosophila* flies tend to walk towards vertical gratings rather than horizontal ones, vertical gratings being capable of generating an optomotor response that counteracts deviations from a straight course, whereas horizontal contours are not (Wehner and Wehner-von Segesser 1973).

In accord with this interpretation is the fact that contour-following is a relatively rigid, stereotyped behaviour, as is the optomotor response, and that it breaks down in the absence of green-contrast. It is known that the bee's optomotor response is mediated primarily by the green channel (Kaiser 1974; Kaiser and Liske 1972). Another movement-evoked behaviour, the movement-avoidance response recently described by Srinivasan and Lehrer (1984), has similarly been shown to be colour-blind and governed mainly by the green receptors.

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