J Comp Physiol A (1997) 180: 235–243

ORIGINAL PAPER

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Discrimination of coloured stimuli by honeybees: alternative use of achromatic and chromatic signals

Accepted: 25 October 1996

Abstract Using a Y-maze experimental set-up, honeybees *Apis mellifera* were trained to a coloured disc presented against an achromatic background. In subsequent tests they were given a choice between the trained disc and an alternative disc that differed either in its chromatic properties, or in the amount of achromatic green contrast that it produced against the background. Tests were conducted in two experimental situations: one in which discs subtended a visual angle of 30° (as viewed by the bee at the decision point in the Y-maze), and another in which the angle was 6.5° or 5° (depending on the experiment). At the visual angle of 30°, the bees' choice behaviour was governed by the differences in chromatic properties, and not by the differences in the amount of green contrast. With the 6.5°- and 5°-discs, on the other hand, it was governed by the differences in the amount of green contrast, and not by the differences in chromatic properties. Consequently, in the present discrimination task, bees use either chromatic or achromatic cues, depending on the visual angle subtended by the stimuli at the eye. Results of a further experiment, in which the trained disc was tested against discs that produced various amounts of green contrast, confirm the above conclusion and show, in addition, that bees learn the green-contrast difference between a trained and a non-rewarded alternative.

Key words Honeybees · *Apis mellifera* · Vision
Chromatic contrast · Achromatic contrast

Introduction

The visual system of the honeybee has been investigated in more detail than that of other invertebrate species. Behavioural (Daumer 1956; von Frisch 1965; Menzel 1967; von Helversen 1972) and electrophysiological studies (Autrum and von Zwehl 1964; Menzel and Blakers 1976; Peitsch et al. 1992) have shown that the bee possesses a trichromatic colour vision system, with three spectral types of photoreceptors peaking in the ultraviolet (344 nm), blue (436 nm), and green (556 nm) regions of the spectrum, respectively [review: Menzel and Backhaus (1991)].

Previous analyses have shown that the discrimination of colours by the honeybee is mediated by two opponent neural subsystems (Backhaus 1991; Menzel and Backhaus 1991; Chittka et al. 1992). An achromatic system, like the luminance channel found in the primate visual system (Shapley 1990), is apparently not involved in the colour choice of bees (Backhaus 1991; Chittka et al. 1992; Brandt and Vorobyev 1997). However, achromatic vision does occur in the bee, namely whenever only one spectral type of receptor is involved in a visual task. For example, E-vector analysis is mediated exclusively by the ultraviolet-sensitive receptor (Wehner and Rossel 1985), and motion detection is mediated exclusively by the long-wave, green-sensitive receptor (Kaiser and Liske 1974). Because a single spectral type of photoreceptor cannot code for colour, these performances are colour blind. As opposed to primates, chromatic and achromatic systems in the honeybee seem to be largely independent. The achromatic signal provided by the excitation difference between target and background perceived by the green receptor, i.e. "green contrast" (Srinivasan and Lehrer 1984), was shown to govern the bee's performance in several tasks that do not involve colour discrimination [reviews: Lehrer (1987, 1993)]. Green contrast was shown to be the major cue used in several tasks that require the use of image motion, such as flight stabilization (Lehrer et al. 1985; Lehrer 1990), distance estimation (Lehrer et al. 1988; Srinivasan et al. 1989), and edge detection (Lehrer et al. 1990).

In a previous paper it was shown that bees use both the chromatic and the achromatic systems for the detection of coloured stimuli (Giurfa et al. 1996). These

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systems were found to have different receptive field sizes: the minimum visual angle required for the detection of a coloured stimulus is 15° if the stimulus presents chromatic contrast but no green contrast (Giurfa et al. 1996), whereas it is 5° if the stimulus presents both kinds of contrast (Lehrer and Bischof 1995; Giurfa et al. 1996). This finding suggests that chromatic stimuli with angular sizes less than 15° loose their chromatic appearance and should thus not be discriminated by the chromatic system. However, they might still be distinguished by the achromatic system if they differ in green contrast. On the other hand, for chromatic stimuli larger than 15°, the differences in both chromatic and achromatic cues could be used for discrimination. This latter hypothesis is obviously incompatible with the notion that colour discrimination in honeybees is mediated exclusively by the chromatic system (Backhaus 1993; Brandt and Vorobyev 1997). However, *direct* evidence for the absence of an achromatic channel in bee colour vision has not been provided experimentally so far. This is because, in the experiments on which this view is based, the angular size of the stimuli, i.e. the distance from which stimuli of a given diameter were chosen, was not controlled for. Moreover, the stimuli were not designed to affect the chromatic and the achromatic systems independently.

The findings by Giurfa et al. (1996) led to the hypothesis that the chromatic systems are involved in the detection task when the stimuli subtend large visual angles at the eye, whereas the achromatic, green-sensitive system is active at small visual angles. The present study was designed to test this hypothesis. Instead of a detection paradigm (Giurfa et al. 1996), we used a dual-choice discrimination paradigm, that enabled us to examine the role of chromatic difference and that of green contrast independently. Bees were trained to a coloured disc that produces a particular contrast against the background. In the tests, the bees were given a choice between the training disc and one that differed from it in either chromatic properties or the amount of green contrast. Chromatic and achromatic properties of the stimuli were designed to affect the chromatic and the achromatic systems independently. In different tests, the stimuli subtend either a large or a small visual angle at the bee's eye. The study further examined the question of whether or not bees learn the magnitude of the green contrast produced by the trained stimulus against the background, or simply choose spontaneously the higher or lower green contrast.

Materials and methods

1. Experimental set-up

Individually marked honeybees, *Apis mellifera carnica,* were trained to enter a Y-shaped, dual-arm apparatus (Fig. 1) to collect 50% (weight/weight) sucrose solution. Entrance to the apparatus was through an open sliding door that was shut as soon as one bee entered the maze. To find access to the arms, the bee had to first pass a central orifice (5 cm in diameter) of a frontal wall, leading to a small "decision chamber" (see Fig. 1). The arms had movable back walls (20 × 20 cm) covered by a grey, achromatic paper. In one of the arms, termed positive, a coloured disc (8 cm in diameter) was presented against the grey background. A bee entering this arm received a reward of sucrose solution when it reached the coloured disc. The reward was provided by an injecting pump mounted behind the back wall. The alternative arm, termed negative, displayed either the grey background only or an alternative coloured disc on the grey background, and offered no reward. The rewarded coloured disc was presented alternately, in a pseudo-random succession, in the right or the left arm, to ensure that bees did not associate the reward with a particular arm. The reward was always associated with the training disc.

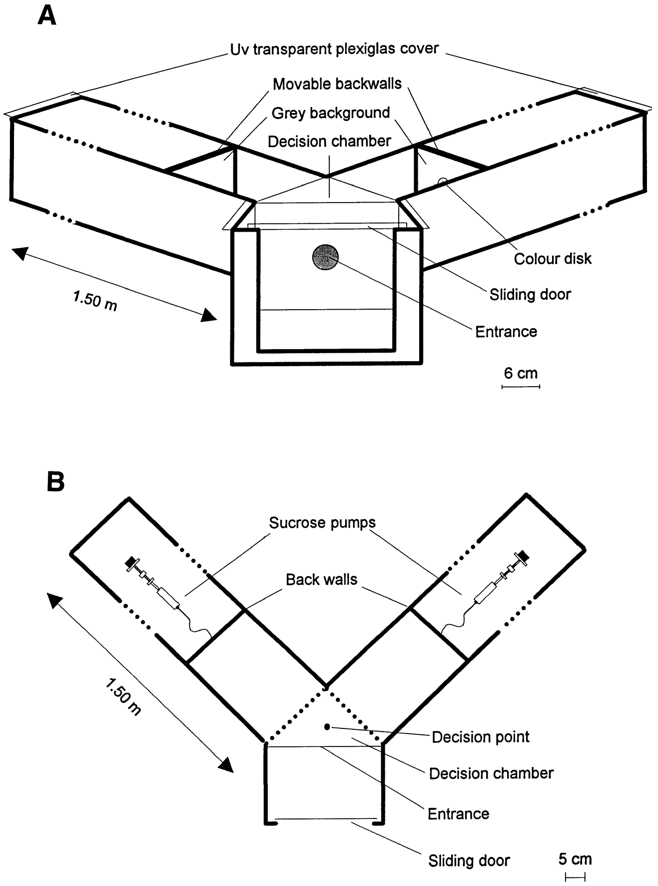

Fig. 1A Frontal view of the Y-maze apparatus. Bees enter the apparatus through a sliding door and face a frontal wall with a central orifice, 5 cm in diameter, through which they must pass to find access to the two arms. Arms had movable back walls covered by a neutral grey paper. Each back wall had a central orifice, 0.5 mm in diameter, through which sucrose solution could be dispensed by means of a cannula connected to an injecting pump. One of the arms, termed positive, presented a colour disc associated with the reward of sucrose solution on a grey background. The other arm, termed negative, presented either the grey background alone or an alternative colour disc. The whole apparatus was covered by an ultraviolet transmitting Plexiglas ceiling. **B** Overhead view of the apparatus

The decision between the two arms could only be made by the bee after it has entered the decision chamber, from which the back walls of both arms could be viewed simultaneously. Thus, the bee's distance from the stimulus, and therefore the visual angle subtended by the latter at the bee's eye as viewed from the decision point, could be controlled for. We defined the decision point as the point being in the middle of the decision chamber (see Fig. 1). Visual angles given correspond to the distances of the targets to this point.

2. Stimuli

The papers used for constructing the discs and the background were either printed using a colour bubble jet printer (Canon BJC 600e) in order to create stimuli of desired spectral characteristics (Experiment 1), or were cut out of HKS-N pigment papers (K+E Stuttgart, Stuttgart-Feuerbach, Germany) (Experiment 2).

Spectral reflectances of the stimuli and background used were measured with a flash photometer (SR01, Gröbel UV-Elektronik, resolution 1 nm) and calibrated against a $BaSO_4$ white standard. From these, receptor-specific contrasts, i.e. the relative number of absorbed quanta q with respect to the background, were calculated as:

$$q_{\mathrm{i}} = \frac{\int_0^\infty I(\lambda)R(\lambda)S_{\mathrm{i}}(\lambda)d\lambda}{\int_0^\infty I(\lambda)B(\lambda)S_i(\lambda)d\lambda}, \; i = \text{uv, blue, green receptor;} \qquad (1)$$

with $I(\lambda)$ being the spectral intensity distribution of the illuminating light (normlight function D65), $R(\lambda)$ the spectral reflectance of the stimulus, $B(\lambda)$ the spectral reflectance of the background and $S_{\mathrm{i}}(\lambda)$ the spectral sensitivity of the receptor with index i (Menzel and Backhaus 1991).

Intensity contrast of a stimulus against the background was calculated as the sum of the absorbed quanta in the three receptor types, relative to the background (see Eq. 1). We tested this achromatic cue, along with receptor-specific contrasts, to analyze the role and incidence of both achromatic cues in our experimental results.

To quantify chromatic differences, the colour space proposed by Backhaus (1991) was used. In such a space, chromatic coordinates A and B of the stimuli were determined as:

$$A = \sum_{i=u,b,g} a_i \frac{q_i}{q_i + 1}, \quad B = \sum_{i=u,b,g} b_i \frac{q_i}{q_i + 1} \qquad (2)$$

with $a_{\mathrm{i}} = \{-9.86,\ 7.70,\ 2.16\}$ and $b_{\mathrm{i}} = \{-5.17,\ +20.25,\ -15.08\}$; i = uv, blue, green receptor.

The perceptual colour distance D between two stimuli S_1 and S_2 was calculated as the sum of the absolute differences in chromatic coordinates A and B [city block metric; see Backhaus (1991, 1993)]:

$$D(S_1, S_2) = |A_{S1} - A_{S2}| + |B_{S1} - B_{S2}| \qquad (3)$$

A prerequisite for a successful training in this behavioural context is that colour stimuli must provide chromatic contrast against the background (Giurfa et al. 1996). All stimuli chosen for the experiments reported in this work provided such a contrast. Chromatic contrast is defined as the perceptual colour distance D of a stimulus (S) to the background (*Back*). Because the background coordinates in the colour space used are (0,0) (Backhaus 1991), chromatic contrast D is calculated as:

$$D(S, Back.) = |A_S| + |B_S| \qquad (4)$$

Colour distance from trained discs, chromatic contrast, receptor specific contrasts and intensity contrast of the stimuli against the backgrounds used in both experiments are given in Table 1. Because only chromatic and green contrasts, but not intensity contrast, influence the detection performance of bees (Giurfa et al. 1996), stimuli were chosen to control for these two specific parameters.

2.1. Experiment 1

Stimuli and grey background were printed by means of the colour printer. The rewarded stimulus (henceforth "standard 1") was yellow to the human observer. The alternative stimuli (henceforth "alternatives" 1 and 2) were light blue and yellow to the human observer, respectively. Figure 2 shows the spectral reflection curves of stimuli and background and their loci in the COC diagram of the honeybee (Backhaus 1991, 1993). The green contrast values of each stimulus relative to the background are also shown (Fig. 2b; inset). The three stimuli were chosen such that the role of chromatic properties and of green contrast could be tested independently.The experiment consisted in training bees to detect and discriminate a standard from two alternatives, one differing chromatically but similar in its amount of green contrast (alternative 1), and another differing in its amount of green contrast but similar in its chromaticness (alternative 2) (see Table 1, Fig. 2).

2.2. Experiment 2

In this experiment, the constant training stimulus (henceforth "standard 2") was cut from a blue HKS-43N paper (K+E Stuttgart, Stuttgart-Feuerbach, Germany). The alternative stimuli were

Table 1 Characteristics of the stimuli used in the present study. Colour distance from standard, chromatic contrast, receptor-specific contrasts for the three receptor types, ultraviolet (UV), blue (B) and green (G), and intensity contrast of the stimuli and backgrounds used in Experiments 1 and 2 (for definitions see Materials and methods). a) Experiment 1; b) Experiment 2

Colour stimulus	Colour distance from standard (COC units)	Chromatic contrast (COC units)	Receptor contrast (absorbed quanta relative to the background)			Intensity contrast (sum of absorbed quanta relative to the background)
			UV	B	G	
a) Experiment 1						
Background	2.7	–	1	1	1	3
Standard 1	–	2.7	1.7	1.5	3.2	6.4
Alternative 1	5.3	2.8	1.8	3.9	3.1	8.8
Alternative 2	0.5	2.6	2.5	2.5	6.4	11.4
b) Experiment 2						
Background	5.0	–	1	1	1	3
Standard 2 (HKS-43N)	–	5.0	1.3	2.9	1.3	5.5
Alternative 1′ (HKS-41N)	1.5	3.5	0.6	1	0.7	2.3
Alternative 2′ (HKS-3N)	13.3	8.3	0.8	0.5	4.1	5.4
Alternative 3′ (HKS-62N)	10	5.0	0.7	0.4	1.4	2.5

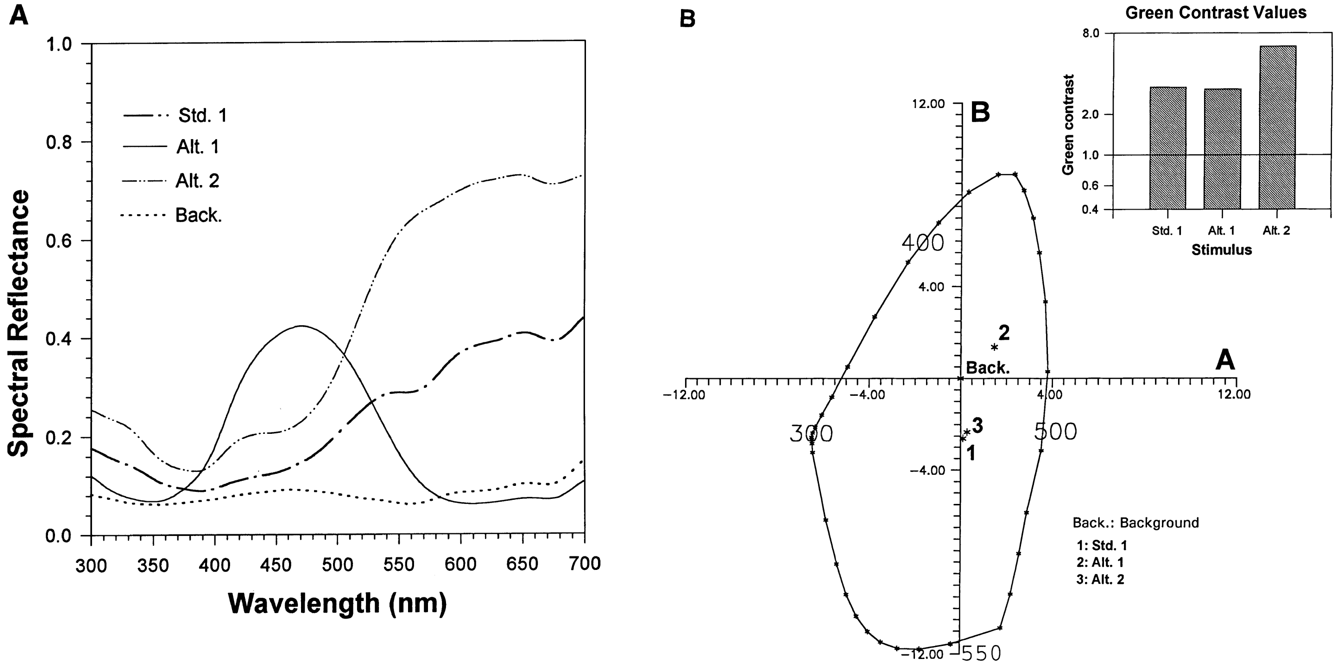

Fig. 2A, B Stimuli used in experiment 1: **A** Spectral reflection curves of standard 1, alternative 1, alternative 2 and background (*Back.*) produced by means of a colour bubble jet printer; **B** loci of the stimuli in the color opponent coding diagram. The diagram represents the cell excitations of two types of colour opponent coding cells, A and B. The origin (*Back.*) represents the grey background. The *solid line* gives the loci of spectral colours in 10-nm steps, and the mixtures of 300 and 550 nm marked in 10% steps. *Inset*: green contrast values for the standard 1 and alternatives 1 and 2

cut from HKS-41N, HKS-3N and HKS-62N papers (henceforth “alternatives” 1′, 2′ and 3′, respectively), to humans dark blue, yellow, and dark green, respectively. The grey background was cut from HKS-92N paper. Figure 3 shows the spectral reflection curves of stimuli and background, and their loci in the COC diagram. The green contrast values of each stimulus against the background are shown in the inset of Fig. 3b. Alternative stimuli were chosen such as to provide either a decrement (alternative 1′) or an increment in green contrast (alternative 2′) with respect to standard 2, or the same amount of green contrast (alternative 3′) as standard 2 (see Fig. 2b, Table 1). Note also that standard 2 and alternative 2′ had the same intensity contrast (see Table1), so that if this parameter were to be used, the two stimuli should be confused by the bees.

3. Procedure

Each experiment began by training a group of marked foragers to enter the Y-maze to collect sucrose solution. The back walls were placed at 20 cm from the entrance hole. Only one experimental bee was present at a time in the apparatus. Recruited bees were excluded by closing the sliding access door. We recorded only the first choice on each visit, because every further choice might be influenced by the outcome of the previous one. The discs were often replaced by new ones to exclude the use of olfactory cues.

Each individual bee was trained to the standard colour disc against the background alone. Bees learned to choose the arm with the standard disc, usually after 10–20 rewards. Subsequently, the bee was trained to discriminate between the rewarded standard disc, and one of the alternative discs. Every time the bee chose the standard it was rewarded and the choice counted as correct. If the bee chose the arm with the unrewarded alternative, the choice was counted as incorrect and the bee was immediately tossed away from the maze. The bee would then enter again the maze and its second choice was not considered. We recorded the bee’s choices until the choice proportion in favour of the standard was significantly higher or lower than 60% (5% level). A maximum of 30 choices (i.e. 30 visits to the apparatus) was recorded for each alternative. Then the alternative colour was replaced by another one and the bee was again trained in this new discrimination task. The sequence of alternative colours was varied randomly from one bee to another. Between the different alternatives, the bee was presented with the original training situation (standard vs. background) to ensure that the original learning level is maintained.

This procedure was performed with the stimuli at two visual angles: a larger one (30°) and a smaller one, close to the bees’ detection limit (6.5° and 5° in Experiments 1 and 2, respectively). The variation of the visual angle was achieved either by placing the back walls at a first distance of 20 cm, and then moving them to a farther one (70 cm in Experiment 1 and 90 cm in Experiment 2), or by changing the diameter of the discs at a fixed distance of 20 cm in order to obtain the angles of 30° and 6.5° (Experiment 1).

4. Statistics

A binomial test was used during the experiment to judge whether or not the stimuli were detectable and discriminable with a probability $P_o > 0.6$. After testing for homogeneity [*G*-test; see Sokal and Rohlf (1981), pp 725–728], results of dual choice experiments of individual bees were pooled and the null hypothesis of a random choice between arms was tested by means of a log-likelihood ratio test (Zar 1984). The same test was used in 2 × 2 contingency tables to analyse whether the response to a particular stimulus changed as depending on the visual angle.

Results

1. Experiment 1

Experiment 1 was designed to test whether bees alternatively use chromatic and achromatic cues depending on the visual angle subtended by the stimuli.

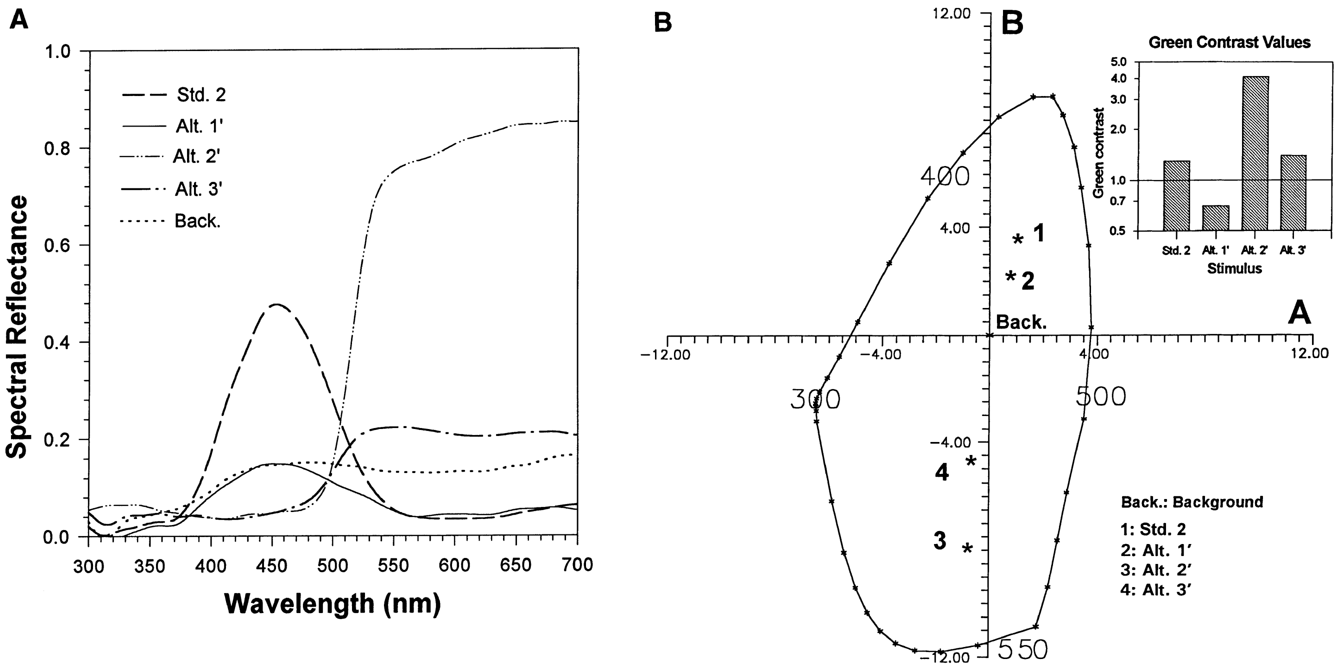

Fig. 3A, B Stimuli used in experiment 2, employing pigment papers. **A** spectral reflection curves of standard 2 (HKS-43N), alternative 1′ (HKS-41N), alternative 2′ (HKS-3N), alternative 3′ (HKS-62N) and background (HKS-92N); **B** loci of the stimuli used in the color opponent coding diagram. For details, see legend to Fig. 2. *Inset*: green contrast values for the standard 2 and alternatives 1′, 2′ and 3′.

Bees tested at the visual angle of 30° (training distance: 20 cm) detected the standard against the background alone and chose it correctly when presented against the chromatically distinguishable alternative 1 (Fig. 4a). If standard 1 was presented against the chromatically similar alternative 2, bees chose randomly between the two stimuli (Fig. 4a). Note that standard 1 and alternative 2 had very different green-contrast and intensity-contrast values (Table 1, Fig. 2b inset), but this did not help the bees to discriminate between them. Thus, neither intensity contrast nor green contrast were used by the bees to discriminate stimuli at this visual angle.

The bees' performance was reversed when the visual angle subtended by the stimuli was close to the detection limit (6.5°; training distance: 70 cm). In this case, bees

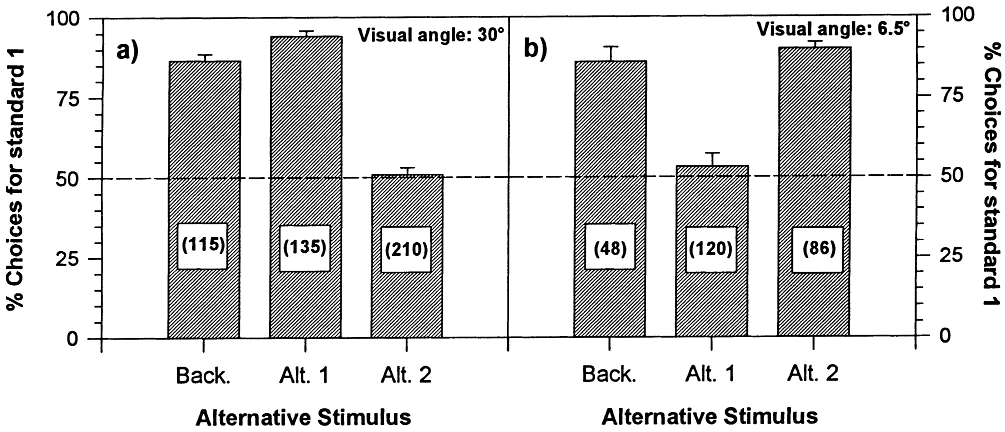

Fig. 4a, b Results of Experiment 1: percentage of choices for the trained standard 1 (mean ± SE) as a function of the alternative stimulus. The *broken line* at 50% indicates random choice level. Values in parentheses indicate the total number of choices recorded in each test situation: **a** visual angle subtended by the stimuli: 30°. *Standard 1 vs. background*: $G = 70.4$; $P < 0.00001$; *standard 1 vs. alternative 1*: $G = 121$; $P < 0.00001$; *standard 1 vs. alternative 2*: $G = 0.1$; NS. $n = 7$ bees; **b** visual angle subtended by the stimuli: 6.5°. *Standard 1 vs. background*: $G = 23.3$; $P < 0.00001$; *standard 1 vs. alternative 1*: $G = 0.5$; NS; *standard 1 vs. alternative 2*: $G = 61.6$; $P < 0.00001$. $n = 4$ bees. From one visual angle to the other, the performance of the bees remained unaltered between tests when the alternative in the negative arm of the maze was the background alone ($G = 0.4$; NS), but changed significantly for alternatives 1 ($G = 57.6$; $P < 0.0001$) and 2 ($G = 43.4$; $P < 0.0001$)

still detected the standard against the background alone (Fig. 4b). However, they were now able to discriminate the standard from alternative 2 that differed from it in green contrast, but not chromatically, and were incapable of discriminating the standard from alternative 1 that differed from it chromatically, but was similar in green contrast (Fig. 4b). Note that, from both achromatic cues available, green contrast and intensity contrast, only green contrast accounts for the bees' choice behaviour: standard and alternative 1 were similar in green contrast but not in intensity contrast (Table 1); thus, the bees' failure to discriminate between these two stimuli shows that green contrast is necessary in this task.

Because the visual angle of the stimuli was varied by moving the back walls of the arms, the variation in visual angle was associated with a variation in distance between the bees' decision point and the targets. Thus, it may be argued that distance, and not visual angle, was the crucial cue for switching from the use of chromatic cues to the use of achromatic ones.

To test this possibility, we repeated the experiment using two different stimulus diameters at the fixed distance of 20 cm. The large targets were the same as before (8 cm in diameter; i.e. stimuli subtended a visual angle of 30°). The small targets had a diameter of 2.3 cm (i.e. stimuli subtended a visual angle of 6.5°). Thus, we reproduced the previous experimental situation without, however, introducing a variation in distance.

Bees tested at the two visual angles (Fig. 5a: 30°; Fig. 5b: 6.5°) behaved consistently with the previous experiment (compare with Fig. 4). Thus, visual angle alone, and not distance, was the crucial cue for switching from the use of a chromatic to the use of an achromatic signal.

2. Experiment 2

This experiment was designed to test whether or not bees learn the amount of green contrast produced by the rewarded disc against the background, or simply choose spontaneously the disc with the higher or the lower green contrast. If they do learn the amount of green contrast of the rewarded stimulus (standard 2), then, at a small visual angle (but not at a large one), the standard is expected to be preferred over alternative 1′ that produces less green contrast, as well as over alternative 2′ that produces more green contrast, whereas alternative 3′, that does not differ from standard 2 with respect to the amount of green contrast, is expected to be confused with the latter.

The results (Fig. 6) show that these expectations are fully met. At a visual angle of 30° (training distance: 20 cm), bees preferred the trained standard over each of the other stimuli (Fig. 6a). Here again, only chromatic differences can account for the bees' choice behaviour. Neither differences in green contrast nor differences in intensity contrast (Table 1) were relevant to this performance. Otherwise, bees should have confused standard with either alternative 3′ or with alternative 2′, respectively.

At the visual angle of 5° (training distance: 90 cm), however, bees preferred the trained stimulus over both alternative 1′ and alternative 2′, but confused it with alternative 3′ (Fig. 6b). Neither chromatic contrast nor intensity contrast play a role in this performance. Only green contrast accounted for the bees' performance. If intensity contrast were used as the guiding achromatic cue, bees should have confused standard 2 and alternative 2′ because both had the same intensity contrast (Table 1). Since this was not the case, our results show

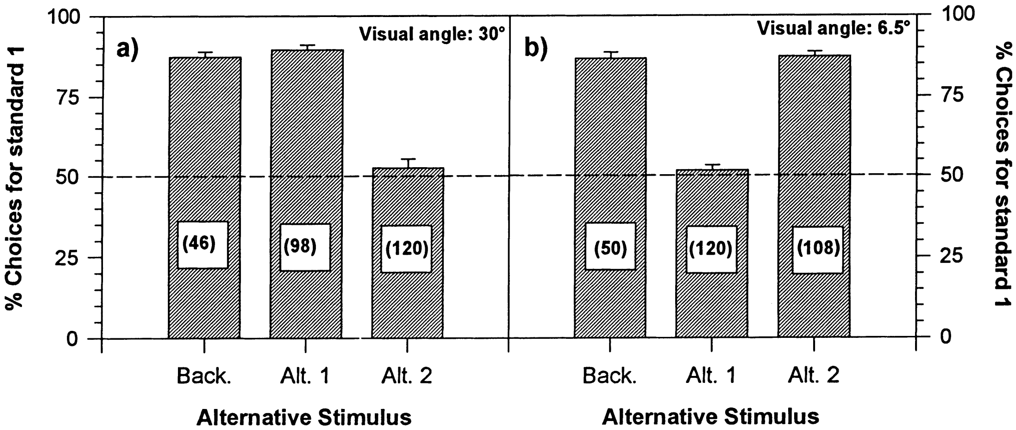

Fig. 5a, b Control experiment: here the visual angle subtended by the stimuli was varied by presenting to the bees two different stimuli diameters at a fixed distance of 20 cm. Percentage of choices for standard 1 (mean ± SE) as a function of the alternative stimulus. The *broken line* at 50% indicates random choice level. Values in parentheses indicate the number of choices recorded in each test situation. **a** visual angle subtended by the stimuli: 30°. *Standard 1 vs. background*: $G = 28.2$; $P < 0.00001$; *standard 1 vs. alternative 1*: $G = 67$; $P < 0.00001$; *standard 1 vs. alternative 2*: $G = 0.3$; NS. $n = 4$ bees; **b** visual angle subtended by the stimuli: 6.5°. *Standard 1 vs. background:* $G = 28.8$; $P < 0.00001$; *standard 1 vs. alternative 1*: $G = 0.1$; NS; *standard 1 vs. alternative 2*: $G = 66.4$; $P < 0.00001$ $n = 4$ bees. From one visual angle to the other, the performance of the bees remained unaltered when the alternative in the negative arm of the maze was the background alone ($G = 0.02$; NS), but changed significantly for alternatives 1 ($G = 37.1$; $P < 0.0001$) and 2 ($G = 33.5$; $P < 0.0001$)

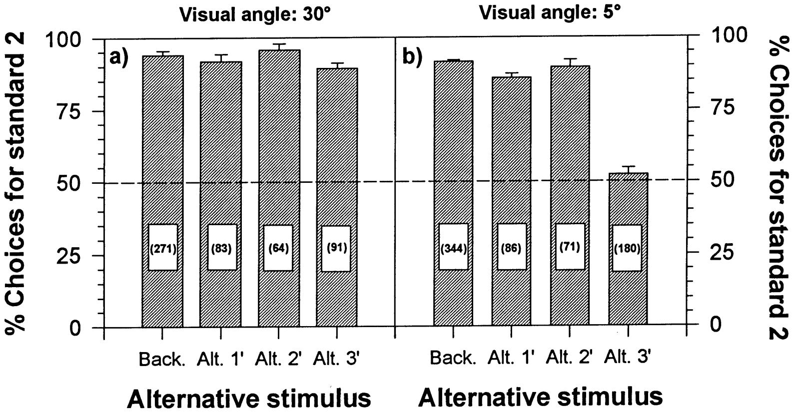

Fig. 6a, b Results of Experiment 2: percentage of choices for the trained standard 2 (mean ± SE) as a function of the alternative stimulus. The *broken line* at 50% indicates random choice level. Values in parentheses indicate the number of choices recorded in each test situation. **a** visual angle subtended by the stimuli: 30°. *Standard 2 vs. background*: $G = 243.3$; $P < 0.00001$; *standard 2 vs. alternative 1′*; $G = 58.1$; $P < 0.00001$; *standard 2 vs. alternative 2′*: $G = 58.8$; $P < 0.00001$; *standard 2 vs. alternative 3′*: $G = 59.1$; $P < 0.00001$. $n = 8$ bees. **b** visual angle subtended by the stimuli: 5°. *Standard 2 vs. background*: $G = 181.2$; $P < 0.00001$; *standard 2 vs. alternative 1′*: $G = 46.2$; $P < 0.00001$; *standard 2 vs. alternative 2′*: $G = 44.4$; $P < 0.00001$; *standard 2 vs. alternative 3′*: $G = 0.4$; NS. $n = 6$ bees. From one visual angle to the other, the bees' performance remained unaltered for all alternative stimuli presented in the negative arm of the maze (*background*: $G = 0.9$; NS; *alternative 1′*: $G = 0.7$; NS; *alternative 2′*: $G = 1.6$; NS) except for alternative 3′ ($G = 37.2$; $P < 0.0001$)

that, at this visual angle, choices were exclusively guided by the achromatic cue provided by green contrast.

These results confirm those obtained in Exp. 1, and show, in addition, that bees learn the green contrast difference between standard and alternative stimulus against the background. The fact that bees were confused only when both stimuli had the same green contrast (standard 2 and alternative 3′) but otherwise always chose the standard, irrespective of whether its green contrast was higher or lower, shows that bees learn to discriminate stimuli on the basis of their green contrast value.

Discussion

Our results show that bees rely alternatively on chromatic or achromatic cues, depending on the visual angle subtended by a colour stimulus. This means that stimuli that are indistinguishable at a particular angular size are easily discriminated at the other and vice versa, depending solely on the existence of chromatic or achromatic differences. When the visual angle subtended by a coloured target at the bee eye is large, chromatic information is sufficient to explain the choice behaviour of free-flying bees. At this visual angle, achromatic cues such as intensity contrast and green contrast are ignored although they are in principle available to the visual system. Thus, the assumption that honeybee colour vision is two-dimensional because it does not involve an achromatic system (Backhaus 1991; Chittka et al. 1992; Brandt and Vorobyev 1997) is demonstrated here for the first time, using stimuli designed to affect the chromatic and the achromatic systems independently (Experiment 1). We further conclude that two-dimensional colour vision is constrained to large visual angles subtended by the targets at the bee eye. At small visual angles, however, bees exclusively use an achromatic cue, namely green contrast, for discriminating between coloured stimuli. Stimulus intensity, which is another potential achromatic cue, is not used in the discrimination task, whether a large or a small visual angle is involved. This finding may explain why intensity contrast, defined as the sum of quanta absorbed by the three spectral receptor types relative to the background, was never found to play a role in honeybee colour vision (Backhaus et al. 1987; Backhaus 1991, 1992; Giurfa 1991; Chittka et al. 1992; Giurfa et al. 1995). The finding that bees use green contrast in the task of discriminating between coloured stimuli of small angular sizes is consistent with results obtained by Giurfa et al. (1996), who investigated the bees' performance in a task involving detection, rather than discrimination of coloured stimuli (Giurfa et al. 1996).

The results of Experiment 2 demonstrate that bees can also learn the difference in green contrast between a trained and an alternative stimulus. Spontaneous reactions towards increased or decreased green contrasts were excluded by the combinations of standard and alternative stimuli chosen. Thus, bees learn to discriminate stimuli on the basis of their green contrast value.

Our results demonstrate the great behavioural flexibility of honeybees in their sensory learning. Clearly, natural food sources are compounds of stimuli offering many cues simultaneously. Although bees may learn different cues in different tasks, they always use the most salient one, guiding them most reliably to the goal (Menzel 1979; Lehrer 1994). This is valid not only for cues belonging to the same sensory modality (e.g. vision), but also for cues related to sensory modalities as different as odour and colour (Giurfa et al. 1994). Thus, it is not surprising that a cue that is not effective in one case is used in another. Green contrast, which is obviously present in the stimuli even when these subtend large visual angles, appears not to be relevant under these conditions, but it is perceived and learned at small visual angles in such a way that it controls behaviour.

Two possible hypotheses may be formulated to explain the finding that green contrast is ignored at large visual angles but learned and used for discrimination at small visual angles. The first hypothesis refers to the learning level and can be called the "Facilitation Hypothesis" (Giurfa et al. 1996). It postulates that chromatic contrast facilitates the learning of green contrast. Because green contrast is available, but not used, at visual angles where the primary association guiding the bees' choice behaviour is that between colour and reward, chromatic contrast may be the salient cue which facilitates the learning of green contrast. Thus, at large visual angles, the primary association would push the green contrast cue above threshold so that it becomes the cue guiding the bees' choice behaviour at near-threshold visual angles.

The second hypothesis that we would like to introduce here refers to the sensory level and can be called the "Angular-Size Tuning Hypothesis". It postulates that the green contrast channel and the colour contrast channel differ in their angular-size tuning so that the chromatic channel does not convey the signals of objects of reduced angular size, whilst the green contrast channel does not convey the signals of objects of large angular size. This would explain why, at large visual sizes, green contrast is ignored, whilst it is learned and used for discrimination at small visual sizes.

Using exclusively green contrast, bees may detect edges (Lehrer et al. 1990). This task is mediated by the perception of absolute motion cues, i.e. by the perception of absolute motion of contrasting edges on the eye. Such cues were obviously present in our design because our stimuli contrasted with the background. Because the perception of absolute motion cues is driven primarily by signals from the green-sensitive photoreceptors (Lehrer et al. 1990), this may be the context in which the achromatic green contrast detection and discrimination that we demonstrated in this paper takes place.

Acknowledgements We thank Natalie Hempel, Peter Kevan, Miriam Lehrer and D. Osorio for suggestions, stimulating discussions and valuable corrections on early versions of the manuscript. Special thanks are due to Miriam Lehrer for her dedication and effort to improve our manuscript. We also thank Josué Núñez for his support in Buenos Aires, Argentina, and Lucas Martínez, Héctor Verna and Fernando Grosclaude for much technical assistance at the campus of the University of Buenos Aires. Martin Giurfa was supported by the Alexander-von-Humboldt Foundation and by the International Foundation of Science (Stockholm, Sweden); Misha Vorobyev was supported by the Deutsche Forschungsgemeinschaft (AZ Me365/20-1).

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