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ORIGINAL PAPER

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Detection of coloured patterns by honeybees through chromatic and achromatic cues

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Abstract We asked whether the detection range of two-coloured centre-surround patterns differs from that of single-coloured targets. Honeybees *Apis mellifera* were trained to distinguish between the presence and absence of a single-coloured disc or a coloured pattern at different visual angles. The patterns presented colours which were either different in chromatic and L-receptor contrasts to the background, equal in chromatic but different in L-receptor contrasts, or vice-versa. Patterns with colours presenting only chromatic contrast were also tested. Patterns with higher L-receptor contrast in its outer than in its inner element were better detected than patterns with a reversed L-contrast distribution. However, both were detected worse than single-coloured discs of the respective colours. When the L-receptor contrast was the same for both elements, the detection range of the two-coloured and single-coloured targets was the same. Patterns whose colours lacked L-receptor contrast were detected just as single-coloured targets of the same colours. These results demonstrate that both chromatic and L-receptor contrasts mediate the detection of coloured patterns and that particular distributions of L-receptor contrast within a target are better detected than others. This finding is consistent with the intervention of neurons with centre-surround receptive fields in the detection of coloured patterns.

Keywords Honeybee · Colour vision · Pattern vision · Detection · Compound eye

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Introduction

The detection of flowers is of fundamental importance in the foraging behaviour of bees. Recently the detectability of uniformly coloured targets has been extensively studied (Giurfa et al. 1996, 1997, 1999; Giurfa and Vorobyev 1998). Since many flowers are not uniformly coloured, but present coloured patterns, we raise the question of how well bees detect coloured patterns.

Numerous studies on the visual system of the honeybee show that this insect has a trichromatic colour vision system (Daumer 1956; von Frisch 1965) with three kinds of spectrally selective photoreceptors, maximally sensitive in the ultraviolet [S (or UV) receptor, $\lambda_{max} = 344$ nm], blue [M (or blue) receptor; $\lambda_{max} = 436$ nm], and green regions of the spectrum [L (or green) receptor; $\lambda_{max} = 544$ nm] (Autrum and von Zwehl 1964; Menzel and Blakers 1976; Peitsch et al. 1992; for review see Menzel and Backhaus 1991). Visual information is processed by chromatic mechanisms mediated by colour opponent (subtractive) interactions between receptor signals. In the visual neuropiles of the bee brain, evidence for these mechanisms has been found (Kien and Menzel 1977; Hertel 1980; Riehle 1981). Visual information is also accessed by achromatic visual pathways. For the honeybee, two types of achromatic pathways have been described: E-vector analysis for navigation is driven by the S-receptor (Wehner and Rossel 1985), whilst the achromatic signal provided by the L-receptor enables bees to perform various visually guided tasks related to motion (Kaiser and Liske 1974;

see review in Lehrer 1993) as well as to recognize orientation in achromatic gratings (Giger and Srinivasan 1996).

For the detection of coloured stimuli, both chromatic and achromatic visual pathways are involved (Giurfa et al. 1996, 1997). The visual pathways have different receptive fields, which are tuned to targets subtending large or small visual angles. The minimum visual angle required for detecting a coloured stimulus is 15° if the stimulus presents chromatic contrast but no L-receptor contrast. It is 5° if the stimulus presents both chromatic and L-receptor contrasts. At visual angles close to the detection limit (5–15°), the choice performance is guided by the L-receptor contrast exclusively (Giurfa et al. 1997; Giurfa and Vorobyev 1998). These results were obtained for the frontal part of the compound eye. Similarly, in the ventral region, the use of chromatic and achromatic cues depends on the visual angle subtended by the stimulus (Giurfa et al. 1999). Although the detection ranges differ for the ventral part of the eye, the same type of neural machinery might be involved in target detection.

We conducted experiments with free-flying honeybees and determined the detection range of concentric coloured patterns. The colours of the patterns were chosen to present a systematic variation of their chromatic and achromatic properties. They were either different in chromatic and L-receptor contrasts, equal in chromatic but different in L-receptor contrasts, or vice-versa. Patterns which presented colours with chromatic contrast and no L-receptor contrast to the background were also used. The results of these experiments enable us to discuss the detectability of natural colour patterns present in flowers (Daumer 1958; Kugler 1963). Such patterns increase, through different spatial arrangements of colour components, the variability of floral displays.

Material and methods

Experimental set-up and procedure

The procedure for these experiments was the same as that used by Giurfa et al. (1996). Individually marked honeybees *Apis mellifera carnica* were trained to enter a dual-arm Y-maze and to collect 50% (weight/weight) sucrose solution. The Y-maze was illuminated by natural daylight. One of the arms presented the rewarded circular stimulus against a grey background on its back wall, whilst the other presented only the grey background. Thus, bees had to distinguish between the presence and absence of the stimulus. Bees could see both back walls only when they were within a decision chamber. In the beginning of each experimental session the back walls were set at 15 cm from the centre of the decision chamber (decision point). The first choice per visit of each individual bee was recorded. Only one bee was present in the maze. After the bee had reached significance in its choices (binomial test), the back walls were moved further away from the decision point, reducing the angular size of the stimulus. If at a certain visual angle significance was not reached, bees were tested until completing 30 choices, and, then, they were tested again with the stimulus located at 15 cm from the decision point to ensure that they still followed the trained rule of flying directly into the arm which presented the stimulus.

Bees could move within the decision chamber before entering one of the arms. The maximal distance from which a bee could see both stimuli exceeded the distance from the centre of the camera by 5 cm, and the minimal distance was shorter than it by 5 cm. We calculated the maximum and minimum angular size of the stimulus for each test distance based on the maximal and minimal distance to the back walls. This allowed us to estimate the error of the visual angle (see Giurfa et al. 1996, 1999). The detection limit was defined by the cross point between the behavioural detection function ('correct choices vs. visual angle of the target') and the statistical criterion of significance ($P_0 = 0.6$). Its angular error was derived by linear interpolation from the error of the visual angle at which detection was still possible and of the next visual angle at which detection was not possible anymore.

Stimuli

The concentric patterns consisted of an inner disc surrounded by a ring that was coloured differently. The patterns were 8 cm in outer diameter. The inner disc was 5.66 cm in diameter. The area covered by each colour in the pattern was the same. The following colours were used: cyan, yellow 1, yellow 2, orange, blue, brown, violet and grey. The coloured papers were produced either by an inkjet printer (Canon BJC 610 for cyan and yellow 1 and the respective grey background) or a laser printer (Apple Color LW 12/660 for orange, yellow 2, blue). For brown and violet we used coloured cardboards HKS 82 N and HKS 33 N, respectively (K + E Stuttgart, Stuttgart-Feuerbach, Germany). The grey background was procured by HKS 92 N. Spectral reflectances of the coloured papers were measured with a flash spectrophotometer (SR01, Gröbel Elektronik, Germany; Fig. 1a, b).

For each part of the pattern we calculated receptor-specific and chromatic contrasts to the grey background and to the reciprocal part of the pattern (Table 1). Receptor-specific contrasts (q_i) were calculated as:

$$q_i = Q^t{}_i / Q^b{}_i, \tag{1}$$

where $Q^t{}_i$ and $Q^b{}_i$ are the quantum catches of receptor i corresponding to target and background colours, respectively.

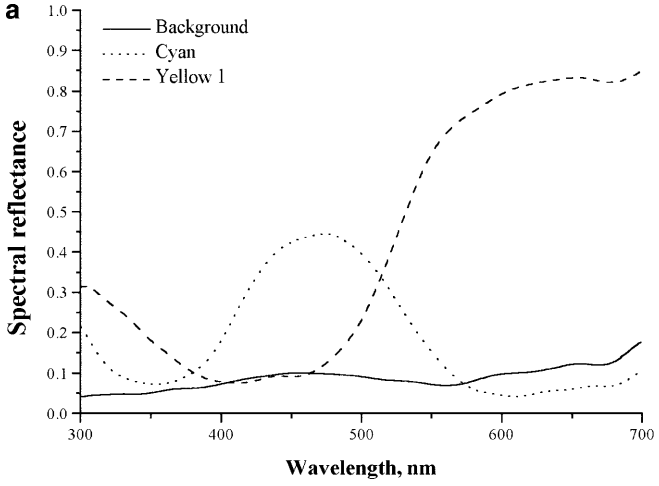
$$Qi = \int_{300}^{700} I(\lambda) Si(\lambda) R(\lambda) d\lambda \tag{2}$$

where i = S,M,L; λ denotes the wavelength, $I(\lambda)$ is the illumination spectrum (standard function D65 in quantum units; see Wyszecki and Stiles 1982), $S_i(\lambda)$ is the spectral sensitivity function of receptor i (Menzel and Backhaus 1991), and $R(\lambda)$ is the reflectance spectrum of the coloured paper.

In our previous publications we calculated the chromatic distance (ΔS) between stimuli in the bee's colour space using the COC model of Backhaus (1991). This model predicts that ΔS decreases with increasing average reflectance of stimuli, thus making bright stimuli difficult to detect, a prediction which is at odds with recent experimental results (Vorobyev et al. 1999; Hempel de Ibarra et al. 2000). Here we use the Receptor Noise Limited model (RN model; Vorobyev and Osorio 1998; Vorobyev et al. 2001) which postulates that colour distance is independent of the average reflectance of the stimuli. Similar to the COC model, the RN model is based on the assumption that colour is coded by two chromatic (colour opponent) mechanisms. In the case of stimuli, which differ only slightly from each other in their average reflectance, both models give practically identical predictions (Vorobyev and Brandt 1997). The distance in the colour space (chromatic distance) was calculated as:

$$\Delta S = \sqrt{\frac{\omega_S^2(\Delta f_L - \Delta f_M)^2 + \omega_M^2(\Delta f_L - \Delta f_S)^2 + \omega_L^2(\Delta f_S - \Delta f_M)^2}{(\omega_S\omega_M)^2 + (\omega_S\omega_L)^2 + (\omega_M\omega_L)^2}}, \tag{3}$$

where ω_i denotes the standard deviation of the noise in the receptor mechanisms i, $f_i = ln(q_i)$ is the receptor signal and Δf_i the difference



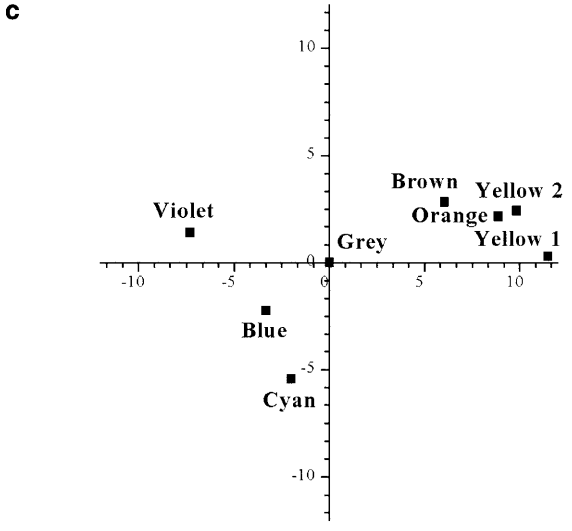


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Fig. 1 Spectral reflectance curves and co-ordinates in the bee's colour space of the grey backgrounds and the coloured papers used in the experiments: **a** cyan-yellow colour pairs (cyan and yellow 1) and their grey background; **b** blue-orange and yellow-orange colour pairs (blue, orange, yellow 2) and their grey background; **c** loci of the colours in the colour diagram representing the bee's colour space. From the relative quantum catch of each photoreceptor the chromatic co-ordinates and distances between stimuli in the colour space has been calculated for each colour according to the Receptor Noise Limited model of honeybee colour vision (RN model, Vorobyev et al. 1998, 2001; see Table 1). The unity distance corresponds to one standard deviation of the noise. The colour locus of the background is per definition at the cross-point of the axes of a colour diagram (0, 0). As other models of honeybee colour vision, the RN model is based on the assumption that colour is coded by two chromatic (colour opponent) mechanisms, but differently postulates that the distance in the colour space is independent of the average reflectance of the stimuli. In general, the larger the distance between colour loci the better distinguishable are colours. orange and yellow 2 have colour loci which lay close to each other. These colours were indistinguishable for bees (see also Fig. 4). All other pairs of colours were well distinguishable for bees

in receptor signals between two stimuli. Table 1 shows the chromatic distances to the background (ΔS_b) for the used colours and between the colours combined in a pattern (ΔS_{St}). The ω_i values were obtained from electrophysiological recordings in single photoreceptor cells (Vorobyev et al. 2001). According to this estimate $\omega_S = 0.13$, $\omega_M = 0.06$ and $\omega_L = 0.12$. Eq. 3 defines ΔS so that the unity distance corresponds to one standard deviation of the noise. The distance corresponding to a threshold, ΔS^t, depends on a decision rule adopted by bees, i.e. on the false alarm rate; $\Delta S^t = 2.3$ corresponds to a false alarm rate of 0.5 (Vorobyev et al. 2001). In reality, behavioural thresholds are variable and discriminability of colours can be improved by summation of signals of individual photoreceptor cells. However, comparison of thresholds with the RN model predictions indicates that in most cases the stimuli are not discriminable if $\Delta S < 2.3$ (Vorobyev et al. 2001).

A two-dimensional colour opponent diagram corresponding to RN model can be obtained by considering a plane, whose co-ordinates are related to receptor signals f_i by

$$X = A\,(f_L - f_M) \quad Y = B\,(f_S - (a\,f_L + b\,f_M)), \tag{4}$$

where $A = \sqrt{\frac{1}{\omega_M^2+\omega_L^2}}$, $B = \sqrt{\frac{\omega_M^2+\omega_L^2}{(\omega_S\omega_M)^2+(\omega_S\omega_L)^2+(\omega_M\omega_L)^2}}$, $a = \frac{\omega_M{}^2}{\omega_M^2+\omega_L^2}$, $b = \frac{\omega_L{}^2}{\omega_M^2+\omega_L^2}$. Euclidean distance in this X-Y plane is equal to that given by Eq. 3. Figure 1c shows the loci of the colours in the colour diagram representing the colour space of the honeybee.

Coloured patterns had different combinations of chromatic and achromatic cues:

1. Colours presented different chromatic and L-receptor contrast. The colours used were yellow 1 and cyan. Yellow 1 yielded a higher chromatic and L-receptor contrast compared to that of cyan.
2. Colours were similar in their chromatic properties but had different L-receptor contrasts. The colours used were orange and yellow 2. Yellow had a higher L-receptor contrast than orange.
3. Colours were different in their chromatic properties but had the same L-receptor contrast. The colours used were the same orange as above combined with blue.
4. Both colours presented effectively zero L-receptor contrast to the background but were different from each other and from the background in their chromatic properties. In this case, brown and violet papers were used.

Each colour pair was tested in a reciprocal arrangement, i.e. each colour was presented either in the central disc or in the surrounding ring of the pattern. Each combination was tested with a different group of bees. Bees were tested at 15 cm, 35 cm, 55 cm and 85 cm from the decision point, i.e. with patterns (outer diameter) subtending a visual angle of 30.0°, 13.0°, 8.3° and 5.4°. Since single-coloured targets without L-receptor contrast have a smaller detection range, the brown-violet patterns were tested at distances different from previous, e.g. 15 cm, 25 cm, 35 cm and 45 cm, i.e. subtending 30.0°, 18.2°, 13.0° and 10.2°, respectively. The test procedure for the pattern with cyan inside and yellow 1 outside differed from the procedure described, because bees were

Table 1 Spectral properties of the colours used in the experiments. Receptor-specific contrasts represent the quantum catches normalised to the grey background for each receptor type. Chromatic distances to background (e.g. chromatic contrast) and between pattern colours were calculated according to the Receptor Noise Limited model (Vorobyev et al. 1998, 2001) and are given in standard units. Colours are not discriminable for bees if $\Delta S < 2.3$

Colour pairs combined to patterns	Chromatic distance (ΔS_b) to background	Chromatic distance (ΔS_{St}) between pattern colours	Receptor-specific contrasts of different colours to background		
			S	M	L
Yellow 1	11.5	14.6	1.7	1.2	5.6
Cyan	5.8		1.5	3.4	2.6
Orange	9.1	1.0	1.2	0.7	2.3
Yellow 2	10.1		2.2	1.2	4.5
Orange	9.1	13.0	1.2	0.7	2.3
Blue	4.0		2.4	3.6	2.3
Brown	6.7	6.7	0.7	0.4	0.9
Violet	7.4		2.4	2.4	0.9

additionally tested to discriminate the trained pattern against alternative colour stimuli.

We also determined the detection limits for single-coloured discs, 8 cm in diameter, using yellow 1, cyan, brown and violet. In a separate experiment we controlled for the spectral properties of orange, blue and yellow 2 as these were the stimuli that presented the same chromatic contrast but different L-receptor contrast (orange and yellow 2) or different chromatic contrast but the same L-receptor contrast (orange and blue). The experiment consisted in training the bees to the orange disc (8 cm in diameter) in the Y-maze. Afterwards, the training stimulus was presented against the blue or the yellow disc, as non-rewarded alternative, at 30.0° and at 5.4°, following the procedure employed by Giurfa et al. (1997). Discrimination of orange from its non-rewarded alternative should vary depending on the visual angle.

Statistics

A binomial test was used to judge whether or not the stimuli were detectable with a probability $P_0 = 0.6$ (Zar 1999); i.e. a stimulus was detected or discriminated by the bee if $P > 0.6$ and not if $P \leq 0.6$ ($\alpha = 0.05$). The choices from each group tested were pooled after testing for homogeneity (χ^2-test).

Results

The single-coloured cyan and yellow 1 discs presented both chromatic and L-receptor contrast. They were detected by the bees at 5.4°, but not at 4.4° (Table 2). Thus, the detection limit of these targets was $4.5 \pm 0.3°$ (Fig. 2). This result is in agreement with previous findings (Lehrer and Bischof 1995; Giurfa et al. 1996, 1999) showing that uniformly coloured targets presenting both kinds of contrasts are detectable down to a visual angle close to 5°.

Two groups of bees were tested with concentric patterns where yellow 1 and cyan were combined. Yellow 1 had higher chromatic and L-receptor contrasts to background than cyan (Table 1). Depending on whether the inner element was yellow 1 or cyan, we obtained different angular detection thresholds.

The pattern with yellow 1 in the centre and cyan in the surrounding ring was detectable at 13.0°, but not at 8.3°. Thus, its detection limit (α_{min}) was $10.0 \pm 1.3°$. The reversed pattern having cyan in its centre and yellow 1 in its surrounding ring was still detected at 8.3°, but not at 5.4°, thus yielding a detection limit of $6.0 \pm 0.5°$. Compared to the single-coloured targets, the detection range of the patterns was reduced although both colour elements presented chromatic and L-receptor contrast. Furthermore, the detection range differed depending on the arrangement of the colours within the pattern. The arrangement with the yellow 1 surround and the cyan centre was better detected (i.e. yielded a smaller detection threshold) than the reversed arrangement although the colours of both patterns were the same.

In order to find out whether the chromatic or the L-receptor contrast determined the detection limit of coloured patterns, we created three special colours: orange, yellow 2 and blue (Table 1). Orange and yellow 2 shared similar loci in colour space, thus being chromatically identical for bees, but had different L-receptor contrasts. Blue and orange differed in their chromatic properties, but presented the same L-receptor contrast. All three colours differed from the background in both chromatic properties and L-receptor contrast. Thus, bees should discriminate orange from blue but not orange from yellow 2 at a large visual angle because they mainly use chromatic cues for discrimination at this visual range (Giurfa et al. 1997). Oppositely, at a small visual angle they should discriminate orange from yellow 2 but not orange from blue, as they use L-receptor contrast for discrimination at this visual range (Giurfa et al. 1997). These predictions were tested separately using single-coloured targets subtending angles of 30.0° and 5.4° (for details see also Giurfa et al. 1997). Bees were first trained to the orange stimulus at 30.0°. After they learned the stimulus (Fig. 3), they were presented with the rewarded training disc against a non-rewarded alternative, blue or yellow 2. At 30.0°, bees were able to distinguish between orange and blue, but not between orange and yellow 2 (Fig. 3). At 5.4° bees distinguished between orange and yellow 2, but not between orange and blue. Thus, the results of this experiment replicated the results of Giurfa et al. (1997) with different colours and guaranteed that the patterns constructed from such colours were indeed

Table 2 Visual angles at which stimuli were last detected (α_{det}) and consecutively not detected anymore (α_{indet}), given with the upper and lower angular limits

	Visual angles (°)	Coloured patterns and targets
1	α_{det}: 11.4 ≤ 13.0 ≤ 15.2 α_{indet}: 9.1 ≤ 10.2 ≤ 11.4	Brown ins./violet outs. Violet ins./brown outs. Brown Violet
2	α_{det}: 11.4 ≤ 13.0 ≤ 15.2 α_{indet}: 7.6 ≤ 8.3 ≤ 9.1	Yellow 1 ins./cyan outs. Yellow 2 ins./orange outs.
3	α_{det}: 7.6 ≤ 8.3 ≤ 9.1 α_{indet}: 5.1 ≤ 5.4 ≤ 5.7	Cyan ins./yellow 1 outs. Orange ins./yellow 2 outs. Blue ins./orange outs.
4	α_{det}: 5.1 ≤ 5.4 ≤ 5.7 α_{indet}: 4.2 ≤ 4.4 ≤ 4.6	Orange ins./blue outs. Yellow 1 Cyan

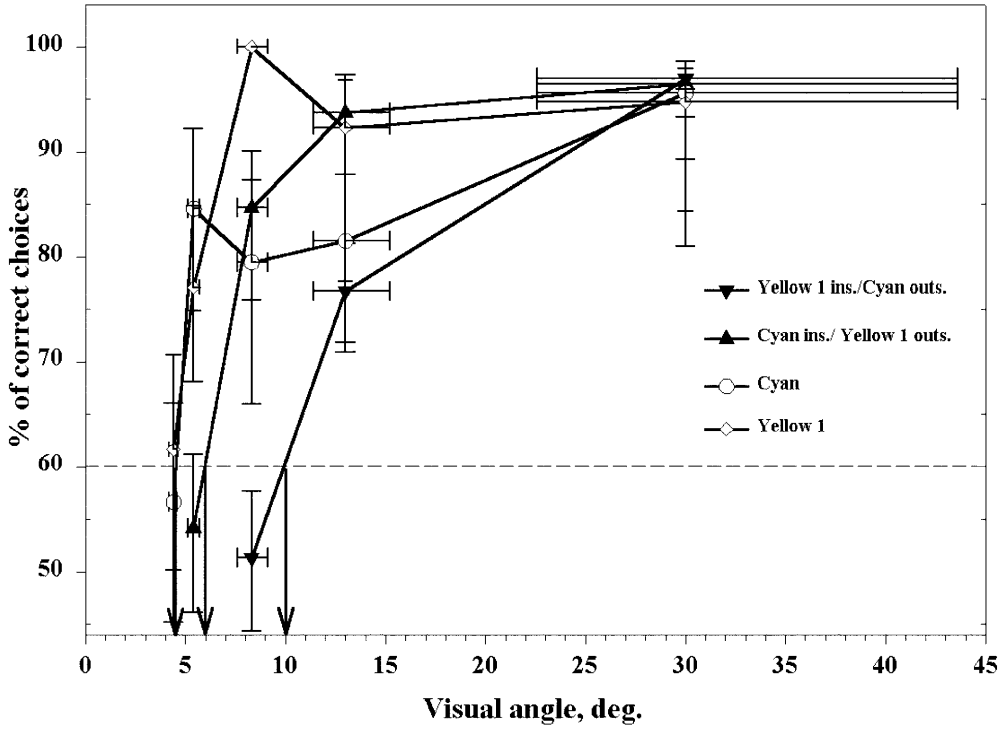


Fig. 2 Detection of coloured patterns presenting different L-receptor contrast of their elements. The minimum visual angle, α_{min}, is given by the cross-point of the behavioural function and the statistical 60% criterion (see *arrows*). *Vertical error bars* show 95% confidence intervals, *horizontal bars* represent the angular error (see Materials and methods). The cyan-yellow 1 patterns yielded lower detection limits than the single-coloured targets [$\alpha_{min} = 4.5 \pm 0.3°$, $n = 3$ bees (yellow 1), $n = 4$ bees (cyan)]. Yellow 1 presented higher chromatic and L-receptor contrast. When yellow 1 was in the centre of the pattern, detection range was strongly impaired ($\alpha_{min} = 10.0 \pm 1.3°$, $n = 8$ bees). The pattern with yellow 1 outside yielded a α_{min} of $6.0 \pm 0.5°$ ($n = 4$ bees)

equated either in their L-receptor contrast or in their chromatic contrast, depending on the colour combination chosen.

The results obtained for patterns constructed with these three colours differed depending on the colours and their spatial arrangement. The orange-yellow 2 patterns appeared chromatically homogeneous to the bees but presented a variation in L-receptor contrast. Their detection ranges were reduced compared to those of the orange-blue patterns which were chromatically inhomogeneous but homogeneous in their L-receptor contrast (Fig. 4). The pattern with yellow 2 inside and orange outside was still detected at 13.0°, but not at 8.3°, thus yielding a detection limit of $9.8 \pm 1.3°$. In contrast, the pattern with orange inside and yellow 2 outside was still detectable at 8.3°, but not at 5.4°, thus yielding a detection limit of $6.4 \pm 0.5°$. Again, the pattern with the highest L-receptor contrast in the surround (i.e. with the yellow-2 ring) was better detectable than its reversed option.

The detection range of both types of orange-blue patterns was similar to that of uniformly coloured targets. The patterns were still detected at 5.4°, but not at 4.4°, yielding a detection limit of $4.5 \pm 0.3°$. As L-receptor contrast is the cue used at the range at which the detection limit is determined, these results confirm that the orange-blue patterns were homogeneous for the bees in this achromatic cue. For the bees, the orange-blue patterns subtending a small visual angle were identical to a single-coloured blue or orange disc

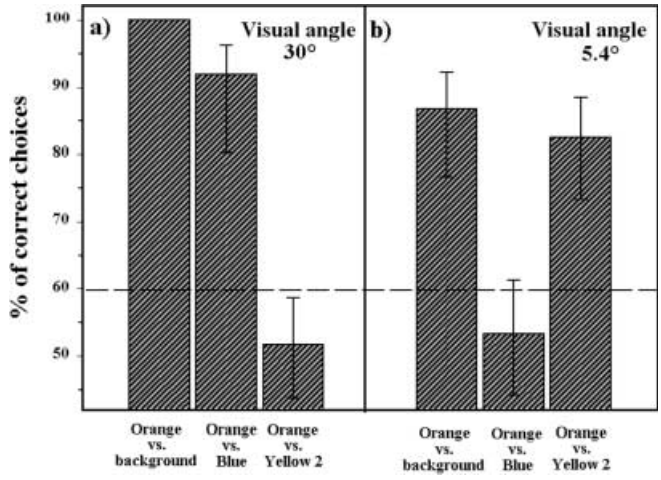


Fig. 3a, b Discrimination of single-coloured targets by chromatic or achromatic cues. Bees were trained to detect an orange disc versus the stimulus-free grey background, afterwards they were confronted simultaneously with the training stimulus and an unrewarded alternative, the blue disc (orange versus blue) or the yellow 2 disc (orange versus yellow 2). Orange and yellow 2 were similar in their chromatic properties, but had different L-receptor contrasts (Table 1). Orange and blue presented the same L-receptor contrast, but were different in colour. The percentage of choices for the rewarded stimulus is shown. **a** At 30.0° bees were able to discriminate orange from blue ($P < 0.0001$, $n = 4$ bees), but not orange from yellow 2 ($P = 0.4$, NS). **b** At 5.4° bees discriminated orange from yellow 2 ($P < 0.0001$), but not orange from blue ($P = 0.3$, NS). *Error bars* show 95% confidence intervals

subtending the same visual angle. Thus, the patterns and the single-coloured discs yielded the same detection limit.

These results indicate that different chromatic contrasts did not affect the detection limit of coloured patterns. Rather, the spatial distribution of L-receptor contrasts drastically affects the detection performance.

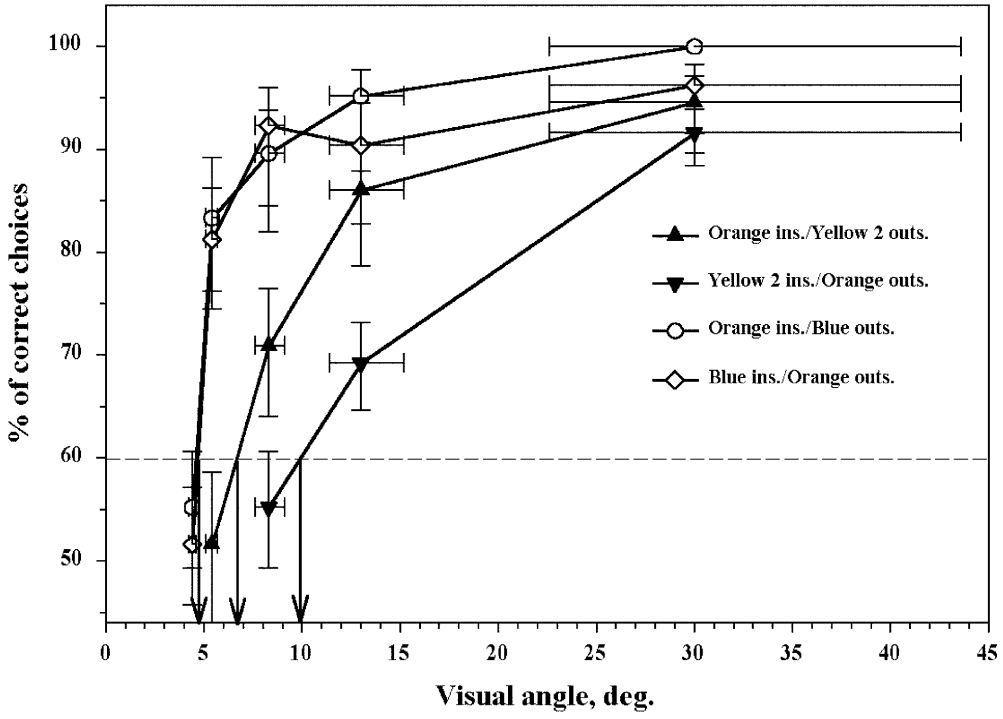
Finally, we tested the detectability of patterns in which the colours (violet and brown) differed in their chromatic properties but had effectively zero L-receptor contrast (0.9) against the background. These patterns allowed us to determine the lower threshold for the chromatic system involved in the detection task. Both pattern types (violet surround-brown centre or brown surround-violet centre) yielded the same detection range: they were detected at 13.0° but not at 10.2° (Fig. 5). The single-coloured brown and violet discs yielded detection ranges that were similar to those obtained for the patterns. They were still detected at 13.0°, but not at 10.2°.

Discussion

Detection of coloured patterns through achromatic cues

The detection limits of the orange-yellow 2 patterns were similar to those of the cyan-yellow 1 patterns. In

Fig. 4 The colours of each pattern were either identical in chromatic properties (yellow 2 and orange) or in L-receptor contrast (blue and orange). The orange-blue pattern yielded the same detection limit as single-coloured targets ($\alpha_{min} = 4.5 \pm 0.3°$, $n = 7$ bees in each group). Yellow 2 presented higher L-receptor contrast than orange (see Table 1). The pattern with yellow 2 inside and orange outside yielded the lowest detection limit ($\alpha_{min} = 9.8 \pm 1.3°$, $n = 14$ bees). The pattern with orange inside and yellow 2 outside was detected from the longer distance than that with yellow 2 inside orange outside but it was detected from a shorter distance than single-coloured targets ($\alpha_{min} = 6.4 \pm 0.5°$, $n = 7$ bees). *Vertical error bars* show 95% confidence intervals, *horizontal error bars* the angular error



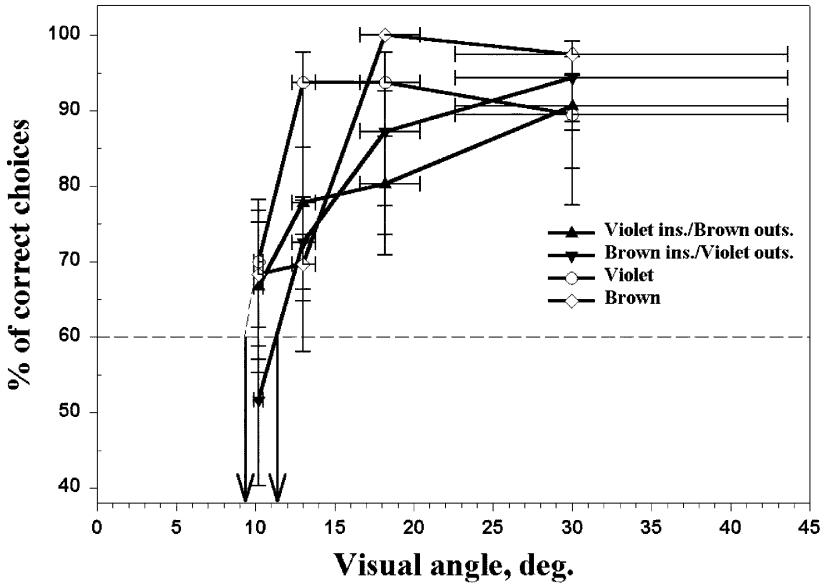


Fig. 5 Detection of coloured patterns and single-coloured targets lacking L-receptor contrast. The stimuli were detected at 13.0°, but not at 10.2° (violet: $P = 0.1$, NS, $n = 3$ bees; brown: $P = 0.1$, NS, $n = 5$ bees; violet inside/brown outside: $P = 0.2$, NS, $n = 4$ bees; brown inside/violet outside: $P = 0.9$, NS, $n = 5$ bees). Thus, the detection limit of these stimuli lays between $9.3 \pm 1.5°$ and $11.2 \pm 1.5°$

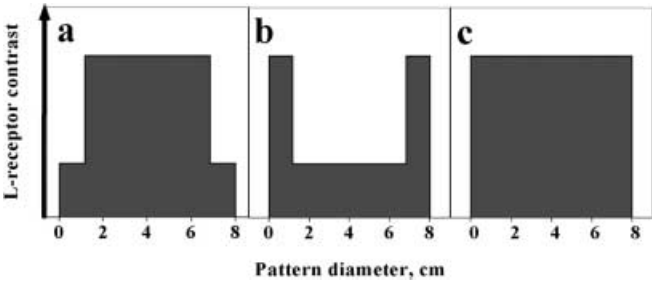


Fig. 6a–c Spatial distribution of L-receptor contrast in the tested patterns. **a** The patterns with yellow 1 or yellow 2 inside and, respectively, cyan or orange outside presented lower L-receptor contrast in the outer ring. These patterns were detected at 13.0° but not at 8.3°. **b** The patterns with cyan or orange inside and, respectively, yellow 1 or yellow 2 outside presented higher L-receptor contrast in the outer ring. These patterns were detected at 8.3° but not at 5.4°. **c** Orange-blue patterns presented the same L-receptor contrast in both pattern elements. These patterns were detected at 5.4° but not at 4.4°

both kinds of patterns one of the elements presented a higher L-receptor contrast (yellow 2 in the orange-yellow 2 patterns and yellow 1 in the cyan-yellow patterns). The patterns had a reduced detection range compared to that of the single-coloured yellow 1 and cyan discs. Thus, the difference in L-receptor contrast between the elements within the pattern influenced the detection range. The detection ranges of both orange-blue patterns were similar to those of the single-coloured yellow 1 and cyan discs. Because orange and blue differed from each other in their chromatic properties, but not in their L-receptor contrast, this result indicates that differences in the chromatic properties of the elements do not affect the detectability of coloured patterns. This result is not surprising if one considers that detectability is evaluated here by determining the detection threshold of patterns presenting L-receptor contrast and that such a threshold is affected by the L-receptor contrast exclusively. Thus, if chromatic properties are not relevant at visual angles close to the detection threshold they cannot affect detectability.

The detectability depended on the distribution of L-receptor contrasts within the patterns (Fig. 6). Both for the cyan-yellow 1 and the orange-yellow 2 patterns, detection was impaired if the L-receptor contrast of the inner disc was higher than that of the outer surrounding ring (Fig. 6a). Detection was improved if the element providing high L-receptor contrast was the outer ring (Fig. 6b). Patterns with uniform L-receptor contrast distribution were most easily detected (Fig. 6c) and were not different in their detection threshold from single-coloured discs of the same colours as the patterns. Clearly, such patterns and the single-coloured discs should have been identical for the bees who evaluated both kinds of stimuli in terms of their L-receptor contrasts at smaller visual angles. In Fig. 7 projections of the patterns having different contrast distributions onto the ommatidia of the bee compound eye are shown. The patterns shown combined yellow 1 and cyan. The lighter grey represents the higher L-receptor contrast of yellow 1 as compared to cyan and background (Table 1). Giurfa et al. (1996) and Ganeshina et al. (1998) showed that a stimulus providing achromatic contrast must cover a certain number of ommatidia in order to be detected. Either seven adjacent ommatidia, or five ommatidia oriented vertically or two ommatidia oriented horizontally were required for detection. However, cyan-yellow 1 and orange-yellow 2 patterns, at small visual angles, were not detected although the number of ommatidia they covered exceeded the minimal number required for detection of uniform targets (compare panels in Fig. 7, last row).

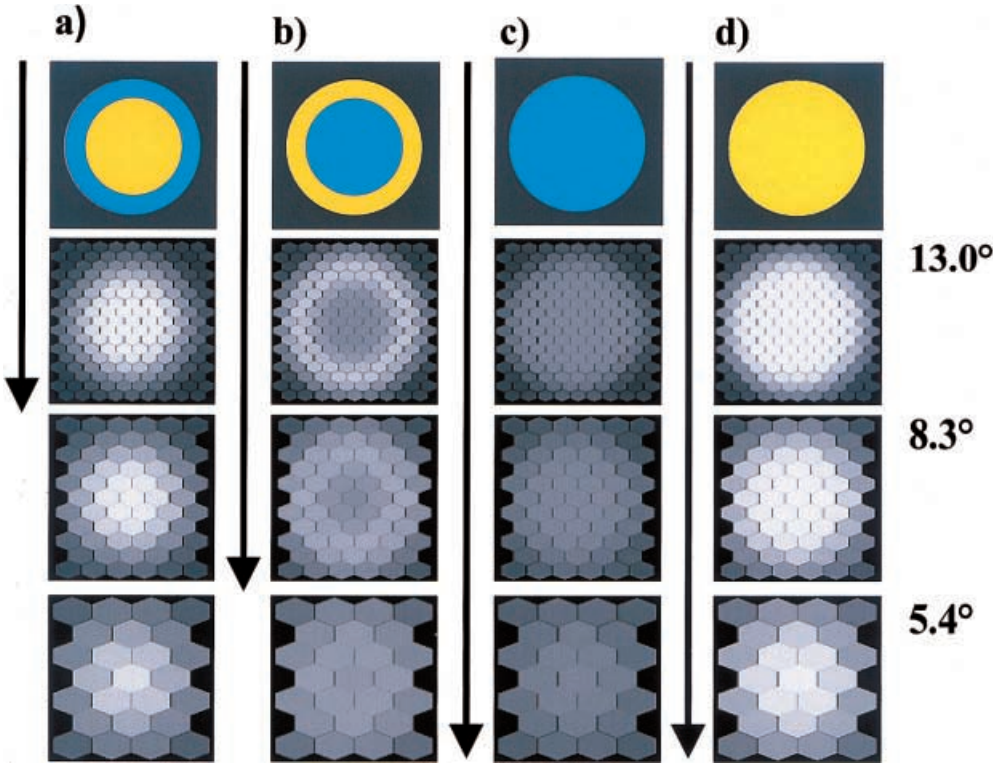


Fig. 7a–d Spatial distribution of L-receptor contrast in cyan-yellow patterns and targets at different visual angles according to the optical resolution of the bee eye. Stimuli were projected onto the ommatidial lattice of the bee eye and the quantum catch for each ommatidium were calculated (for details see Vorobyev et al. 1997). The acceptance angle ($\Delta\rho$) for the frontal part of the bee worker eye is 2.6° (Laughlin and Horridge 1971), the vertical and horizontal interommatidial angles ($\Delta\Phi$) are 0.9° and 1.6° (Kirschfeld 1973). The amount of L-receptor contrast is labelled by different grey levels in the stimuli: yellow 1 has twice the L-receptor contrast than cyan. Column **a** shows the projection of the pattern with yellow 1 inside and cyan outside, **b** the pattern having yellow 1 outside and cyan inside, and columns **c** and **d** the two uniformly coloured disks (cyan, **c**; yellow 1, **d**). Projections are shown for stimuli subtending 13.0° (second row), 8.3° (third row) and 5.4° (last row). The *arrows* indicate the angles at which stimuli were detected by bees

Based on previous experimental results, the existence of detector units with a receptive field in the L-receptor mediated pathway was proposed (Giurfa and Vorobyev 1998). Such detectors may have vertically and horizontally oriented receptive fields (Ganeshina et al. 1998). A simple linear model describes the detector properties (Giurfa and Vorobyev 1998). The width of the central part of the receptive field was estimated to be twice the width of the angular sensitivity function of a single ommatidium and, thus, the signals of several adjacent ommatidia must interact. The model describes well the behavioural data obtained by Giurfa and Vorobyev (1998), where the detection range of a single-coloured target, having only L-receptor contrast and no chromatic contrast, was tested. The upper threshold for the detection of that stimulus was between 10° and 15°, and the lower threshold between 5° and 3°.

Neurons with centre-surround receptive fields are tuned to detection of borders. This may explain the fact that patterns with a higher L-receptor contrast at the outer ring are better detected than those having a higher contrast in the centre of the pattern. It is important to note that due to the low optical resolution of the bee eye the borders of the stimuli are blurred (Fig. 7) and, thus, cannot be resolved from a long distance. The patterns with higher L-receptor contrast at the outer ring have enhanced borders (Fig. 6b); thus, they may provide strong input to the centre-surround neurons, which may improve the detectability. On the other hand, if the L-receptor contrast of the outer ring is low compared to that of the inner disc, the borders of the stimulus are concealed (Figs. 6a, 7), and the detectability may be impaired. While the difference in detectability between the patterns whose elements have L-receptor contrast to each other agrees nicely with the assumption that detection is mediated by linear neurones with centre-surround receptive fields, this simple model does not explain why uniform stimuli are detected at a further distance than those having enhanced borders. From these results and analysis we conclude that non-linear processing of achromatic information is also involved in the detection of coloured stimuli (see also Giurfa and Vorobyev 1998).

Detection of coloured patterns through chromatic cues

In the case of two-coloured patterns, which presented only chromatic contrast to the background but no L-

receptor contrast (brown-violet), the detection limit did not depend on the distribution of colours within the pattern, and was similar to that of single-coloured targets lacking L-receptor contrast (brown or violet). This result implies that detection by means of chromatic vision depends only on the presence of chromatic contrast, not on the distribution of colours within the pattern.

In the case of single-coloured targets presenting L-receptor contrast to the background the stimuli were last detected at 5.4°, but not at 4.4°. Such a detection threshold is identical to that found by Giurfa et al. (1996, 1999) for single-coloured stimuli presented vertically. In our work, all targets lacking L-receptor contrast were detected at 13.0°, but not at 10.2°, while Giurfa et al. (1996) found that circular targets lacking L-receptor contrast were detected at 16.3° and not at 13.0°. Thus, the detection range established in the present study for targets lacking L-receptor contrast is similar, but not identical, to that established previously. Although the difference is small, it is significant and allows an extension of the range of chromatic vision such that its lower threshold now coincides with the upper threshold of achromatic, L-receptor based vision (between 15° and 10°; Giurfa and Vorobyev 1998). Thus, both achromatic and chromatic channels are tuned as sequential systems. This result also implies that the capability of bees to detect stimuli in the applied experimental conditions varied slightly. The reasons for such variations are unclear. They may be attributed to seasonal or motivational variations in different years.

Flowers present different kinds of coloured patterns. Some of them are very tiny, the so-called nectar or pollen guides. Such details cannot be resolved by the bee eye, even at small distances from the flower (Vorobyev et al. 1997), but may orient a bee towards the pollen or nectar after it has landed. Stamens with the pollen or the inner part of petals may form a larger area which is coloured differently than the outer part of the petals, resulting in the formation of common concentric patterns (Kevan 1978). Such patterns could be resolved by a bee during the approach to the flower. Our results indicate that it would be advantageous for a flower to combine colours with certain spectral properties and in a certain spatial arrangement in order to be better detectable by bees. Menzel et al. (1997) showed for the Israeli desert and Mediterranean flora that most of the species analysed had supra-threshold chromatic and achromatic, L-receptor contrasts, as estimated from results of different behavioural and electrophysiological work (Giurfa and Vorobyev 1997; Vorobyev and Brandt 1997; Vorobyev and Osorio 1998). Thus, assuming that flowers usually present L-receptor contrast, the question should be raised, whether the distribution of L-receptor contrasts as naturally available on flower corollas is optimised for detection by bees. Indeed, looking at flowers presenting centre-surround coloured patterns, there is usually a variation of chromatic and L-receptor contrast in the pattern elements (Hempel de Ibarra 2000). The quantitative analysis of the found variation in chromatic and achromatic contrasts will provide a further example of the bee's visual ecology.

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