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Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts

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Abstract Honeybees Apis mellifera were trained to distinguish between the presence and the absence of a rewarded coloured spot, presented on a vertical, achromatic plane in a Y-maze. They were subsequently tested with different subtended visual angles of that spot, generated by different disk diameters and different distances from the decision point in the device. Bees were trained easily to detect bee-chromatic colours, but not an achromatic one. Chromatic contrast was not the only parameter allowing learning and, therefore, detection: \( z_{\text{min}} \), the subtended visual angle at which the bees detect a given stimulus with a probability \( P_0 = 0.6 \), was 5° for stimuli presenting both chromatic contrast and contrast for the green photoreceptors [i.e. excitation difference in the green photoreceptors, between target and background (green contrast)], and 15° for stimuli presenting chromatic but no green contrast. Our results suggest that green contrast can be utilized for target detection if target recognition has been established by means of the colour vision system. The green-contrast signal would be used as a far-distance signal for flower detection. This signal would always be detected before chromatic contrast during an approach flight and would be learned in compound with chromatic contrast, in a facilitation-like process.

Key words Honeybees · Apis mellifera · Colour vision · Detection

Introduction

Apart from enabling flower recognition and discrimination among different flower species (Faegri and van der Pijl 1978; Kevan and Baker 1983; Menzel 1985; Chittka and Menzel 1992), flower colours are considered to be long-distance signals, enabling the far-distance detection of the flowers to be pollinated and exploited as potential food sources by diurnal pollinators like honeybees (von Frisch 1965, p. 521; Kevan and Baker 1983; Chittka and Menzel 1992). Clearly, flowers that are detectable by pollinators from the furthest distance should have a reproductive advantage. However, what 'long-distance' means is mostly unclear and poorly defined. The question of the distance necessary for the effective detection of a flower by a pollinator was first raised in 1933 by Kugler, who released bumblebees in a room with diffuse light and tried to determine the distance at which their flight path straightened out and oriented to black, artificial flowers of known diameter (Kugler 1933), presented on stems, 10–13 cm height. His results show that the greater the diameter of the artificial flowers, the greater the distance from which they are detected. This finding has a simple explanation: an object can be detected when it subtends a visual angle equal to or greater than a limiting angle \( z_{\text{min}} \). Thus, the stimuli, irrespective of their sizes and distances, are likely to influence behaviour in the same way if they subtend the same visual angle. Though Kugler reported only distances for each diameter he tested, his results quantitatively agree with this hypothesis \( z_{\text{min}} = 6.7 \pm 0.7 \) deg (mean \( \pm \) S.E.; \( n = 5 \); calculated from Kugler's tables of distances 1 and 2). His original question can thus be reformulated, i.e., rather than asking for the distance at which an object of given diameter can be detected, one can ask for the angle that an object of given diameter should subtend in order to be detected. To estimate \( z_{\text{min}} \), it is necessary not only to determine the angles at which an animal can still detect an object, but to go
bees at the feeding place in colour discrimination experiments (Daumer 1956; Menzel 1967; von Helversen 1972; Backhaus et al. 1987; Backhaus 1991; Brandt et al. 1993; Chittka et al. 1992). In fact, colour vision of bees appears to be perceptually two-dimensional, in which both dimensions are related to the chromaticity of the stimuli, and excludes brightness as a perceptual dimension (see Backhaus 1993; Menzel and Backhaus 1991). Does this mean that overall intensity- or specific receptor contrasts are also not used as a signal to detect flower colours at a distance at which colour detection might not be effective anymore?

Here we attempt to answer this question by examining how parameters like chromatic contrast (measured as the perceptual distance between a colour stimulus and its background), overall intensity contrast (measured as the difference between a stimulus and its background in the sum of the excitations of all photoreceptor signals), and contrasts to particular colour receptor types (measured as the difference between a stimulus and its background in the excitation for a particular receptor type considered) affect the detectability of coloured stimuli.

Materials and methods

Apparatus

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. The Y-maze, similar to that used by Srinivasan and Lehrer (1988), was situated near a large, open window, in a laboratory room which was well illuminated by natural daylight. The apparatus was covered with an ultra-violet transmitting plexiglas ceiling. Bees entered the room through the open window. They entered the apparatus through the open sliding door and found a frontal wall with a central orifice, 5 cm in diameter, through which they should pass to find access to the two arms.

The arms were 1.50 m long and 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 was dispensed by means of a cannula connected to an injecting pump. The pump consisted of a syringe with a piston driven by a synchronotor, so that a controlled rate of sucrose solution (20 μl/min) was obtained.

Both arms presented a disk of the same colour and diameter in the middle of their back walls. In one of the arms, termed positive, this stimulus was exposed and associated with a reward of sucrose solution. In the other arm, termed negative, the disk was covered by the same grey paper used as a background for the stimulus. The positive and negative arms were interchanged after one or two consecutive rewards to ensure that the bees did not associate the reward with one particular arm. To interchange the arms, the stimulus on the positive wall was covered with the grey cover and the sucrose pump was turned off; the cover of the negative arm was removed, thus exposing the colour disk, and the sucrose pump of this arm (now positive) was then turned on.

The bees were not able to see the stimulus before they entered the apparatus. Thus, they were forced to make a decision within a "decision chamber" (Fig. 1). Within this chamber, they were able to see the stimulus in one arm and the stimulus-free background in the other arm simultaneously. This allowed us to check the distance at which detection of the stimuli, if any, took place. The actual distance (L) from the decision point to the stimuli lay between L₁ and L₂, as shown in Fig. 1B.

Stimuli

The colour disks were 4.6 or 8 cm in diameter. They were cut from HKS-3N, HKS-8N, HKS-33N, HKS-41N, HKS-42N, HKS-82N and HKS-21N papers (K + E Stuttgart, Stuttgart-Feuerbach, Germany) and were bee-green, bee-ultra-violet-green 1, bee-ultra-violet-blue, bee-blue 1, bee-blue 2, bee-ultra-violet-green 2 and bee-aehromatic; respectively (human appearance yellow, orange, violet, dark blue, blue, brown and pink, respectively) (for terminology see Chittka et al. 1994). The grey background was cut from HKS-92N paper and was bee aehromatic. The spectral reflectances of the stimuli were measured with a flash photometer (SR10 Grobel UV Elektroniks resolution 1 nm) and calibrated against a BaSO₄ white standard. Figure 2 shows the spectral reflectance curves of our colour stimulus and background and their loci as calculated according to the colour opponent coding (COC) diagram of the honeybee (Backhaus 1991, 1993). The basis of such a representation of colour is that signals generated by the three spectral photoreceptor types of the honeybee are assumed to be evaluated in two spectrally opponent neural mechanisms which lead to two perceptual dimensions of colour vision. Thus, colours can be represented in a two-dimensional diagram using the two scales assigned to the respective spectrally opponent mechanisms as axes. Calculations were done using the spectral sensitivity curves of a typical, green-sensitive, bee reported by Menzel and Backhaus (1991) for the honeybee worker. The colour difference between two stimuli is calculated as the sum of the absolute differences of the corresponding scale values on the two axes, according to a city-block metric (Backhaus 1991). The adaptation light is assumed to be the spectral reflection of the grey background (B), illuminated by the daylight norm-function D65. The colours used have different perceptual distances to the background (i.e. different chromatic contrasts) as well as different intensity contrasts values and receptor-specific contrasts (Table 1).

The assumption that the intensity perceived from a given light stimulus is based on the summed excitations of all three spectral types of insect photoreceptors has been applied by several authors in the past (Backhaus and Menzel 1987; Backhaus 1991; Menzel and Backhaus 1991) and has proved valid, at least for the bee's phototactic response (Menzel and Greggers 1985). Contrast to a specific receptor type was calculated as the excitation of that receptor produced by a given colour stimulus, relative to that produced by the background (Srinivasan and Lehrer 1988).

Procedure

Each experiment began by training a group of 4-5 marked foragers to enter the Y-maze to collect sucrose solution. The back walls were 20 cm from the entrance hole (control distance). After the bees had learned to respond correctly at this distance, we chose one individual and the others were caged for later use. The learning criterion was that bees chose the colour disk of a given diameter, with a percentage of correct choices greater than 60%, which was achieved usually after 10-20 rewards (at the training distance, and independently of the disk diameter, bees normally reached a percentage of at least 85% correct choices). Only one experimental bee was present at a time in the apparatus. New recruits were excluded by closing the sliding access door. We recorded only the first choice at each visit to avoid the possibility that the second choice might be influenced by the result of the previous choice. The disks were often replaced by new ones to avoid orientation by means of odours.

We recorded the bee's choices until significance (5% level, see below) in favour of the positive arm was obtained. The visual angle subtended by the rewarded colour disk was varied by changing the
a step further and determine the angle at which this object cannot be detected. Clearly, $x_{\text{min}}$ lies between these two angles.

In honeybee workers, recent findings indicate that $x_{\text{min}}$ depends on the intensity contrast to the background (Lehrer and Bischof 1995). Using black or grey disks presented on a white background, so that different intensity contrasts were obtained, Lehrer and Bischof showed that resolution deteriorates as contrast decreases. Moreover, by repeating their experiments with two kinds of green and violet combinations, they were able to show that the overall intensity contrast may not be relevant for detection of model flowers when chromatic contrast is present.

When considering the significance of contrast in the detection task, one may ask how parameters like chromatic contrast (measured as the perceptual distance of a colour stimulus to the background), overall intensity (measured as the sum of all photoreceptor signals), and contrasts to particular colour receptor types, relative to the background, affect the detectability of coloured stimuli by free-flying honeybees. The role of such parameters in object detection by bees is particularly interesting. Whereas receptor-specific contrast has been found to be critically involved in tasks related to motion detection (which is mediated by the green receptor type; see review in Lehrer 1994), polarized light response (mediated by the ultraviolet receptor type; see Rossel and Wehner 1986; Rossel 1993) and phototaxis (mediated by all three receptor types, ultraviolet, blue and green; see Menzel and Greggers 1985), overall intensity contrast has been found to be ignored by

**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 were 1.50 m long and 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 was dispensed by means of a cannula connected to an injecting pump. One of the arms, termed positive, presented a colour disk associated with the reward of sucrose solution. The other arm, termed negative, presented only the grey background. The whole apparatus was covered by an ultraviolet transmitting plexiglass ceiling. **B** Overhead view of the apparatus. $L_1$ is the distance from the entrance hole to the stimulus; $L_2$ is the distance from the end of the decision chamber (10 cm from the entrance hole) to the stimulus. The distance $L$ lies between $L_1$ and $L_2$. $L$ was used for calculating the angle subtended by the stimulus; $L_1$ and $L_2$ were used for the calculation of its upper and lower limits. Further details in text.
bees at the feeding place in colour discrimination experiments (Daumer 1956; Menzel 1967; von Helversen 1972; Backhaus et al. 1987; Backhaus 1991; Brandt et al. 1993; Chittka et al. 1992). In fact, colour vision of bees appears to be perceptually two-dimensional, in which both dimensions are related to the chromaticity of the stimuli, and excludes brightness as a perceptual dimension (see Backhaus 1993; Menzel and Backhaus 1991). Does this mean that overall intensity- or specific receptor contrasts are also not used as a signal to detect flower colours at a distance at which colour detection might not be effective anymore?

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We recorded the bee's choices until significance (5% level, see below) in favour of the positive arm was obtained. The visual angle subtended by the rewarded colour disk was varied by changing the
the procedure with several distances until we reached the distance where significance could not be obtained; in this case, we explicitly tested if the colour stimulus was indeed undetectable.

A binomial test was used to judge whether or not the stimuli were detectable. Statistical methods allow us to check whether an arm is chosen with a frequency $P$ greater than a certain probability $P_0$ (i.e. to reject the null hypothesis $P < P_0$) or smaller than $P_0$ (i.e. to reject the null hypothesis $P > P_0$). Obviously, the disk is not detectable if $P = 0.5$. However, because in dual-choice experiments $P$ lies between 0.5 and 1, it is impossible to reject the hypothesis $P > 0.5$ with a finite number of choices. To overcome this problem, we assumed that the stimulus was detectable if $P > 0.6$ and not detectable if $P < 0.6$ (significance at the 5% level). If significance was not attained after thirty or more choices, we established the probability of $P > 0.6$ and $P < 0.6$. Thus, for each bee trained to forage on a disk of a given diameter and colour, the smallest visual angle that was sufficient for stimulus detection, $x_{min}$, and the maximum visual angle at which detection was impossible, $x_{max}$, were determined. The visual angle was calculated as:

$$x = 2\arctan(D/2L)$$

where $x$ is the visual angle subtended by a colour disk of diameter $D$ at the distance $L$ from the decision point ($L_1 < L < L_2$; see Fig. 1B). We established $x_{min}$ and $x_{max}$ for each particular colour stimulus. $L_1$ and $L_2$ were used for the calculation of their lower and upper limits. $x_{min}$, the limiting visual angle at which the bee detects a stimulus with a probability $P_0 = 0.6$ is defined as:

$$x_{min} = (x_{det} + x_{max})/2$$

Confidence intervals for the choice frequencies measured were calculated using a relationship between the F distribution and the binomial distribution (see Zar 1985, p. 378).

**Results**

According to the colour opponent theory of honeybee colour vision (Backhaus 1991, 1992, 1993), six of our stimuli (bee-ultraviolet-blue, bee-blue [$\times 2$], bee-green and bee-ultraviolet-green [$\times 2$]) were bee-chromatic because their colour difference to the background was sufficient to make them discriminable (range: from 3.7 to 8.3 COC units; see Table 1 and Fig. 2b) and one (human pink) was bee-achromatic (0.1 COC units; see Table 1 and Fig. 2b) and thus differed only in its overall intensity from the background. In agreement with former findings (see Introduction), the coloured stimuli were learned well and were easily discriminated from the background. The achromatic stimulus was not. At the first distance of 20 cm, which corresponded to a visual angle of $30^\circ$ for the 8 cm disk, practically all bees (92.4% ± 4.9; $n = 6$; Mean ± S.E.) learned to follow and to detect the bee-chromatic stimuli (Table 2: $B_1/B_2$; proportion of bees that effectively learned the training stimulus from the total number of bees trained with this stimulus). As opposed to this, training to the bee-achromatic stimulus was difficult, and even after more than 40–50 learning trials, only 2 out of 19 bees were able to learn it. Moreover, these two bees were not consistent throughout the experiment, i.e. they managed to solve the task with the 8 cm diameter disk but not with the 6 cm disk, even if the visual angles presented by the 6 cm disk were the same as for the 8 cm disk.
Table 1. Contrasts of the colour stimuli to the neutral grey background (HKS-92N). Chromatic contrast is expressed in COC units (see text for further details) and represents the perceptual distance from each stimulus to the background. Receptor as well as overall intensity contrasts are expressed in percentages in relation to the excitations produced by background. Positive values represent excitation increments and negative values represent excitation decrements. Overall intensity contrast is the contrast between stimulus and background in the sum of excitations of the three receptor types, ultraviolet (UV), blue (B) and green (G).

<table>
<thead>
<tr>
<th>Colour</th>
<th>Chromatic contrast (COC units)</th>
<th>Receptor contrast (%)</th>
<th>Overall intensity contrast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV contrast (%)</td>
<td>B contrast (%)</td>
<td>G contrast (%)</td>
</tr>
<tr>
<td>Uv-blue</td>
<td>3.7</td>
<td>38.3</td>
<td>43.9</td>
</tr>
<tr>
<td>(HKS-33N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue 1</td>
<td>4.6</td>
<td>-27.1</td>
<td>0.4</td>
</tr>
<tr>
<td>(HKS-41N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue 2</td>
<td>5.0</td>
<td>12.0</td>
<td>48.8</td>
</tr>
<tr>
<td>(HKS-33N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green (HKS-3N)</td>
<td>8.3</td>
<td>-10.2</td>
<td>-36.5</td>
</tr>
<tr>
<td>Uv-green 1</td>
<td>5.4</td>
<td>-0.7</td>
<td>-29.1</td>
</tr>
<tr>
<td>(HKS-8N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uv-green 2</td>
<td>4.0</td>
<td>-20.6</td>
<td>-39.5</td>
</tr>
<tr>
<td>(HKS-82N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Achromatic</td>
<td>0.1</td>
<td>42.7</td>
<td>41.6</td>
</tr>
<tr>
<td>(HKS-21N)</td>
<td></td>
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</table>

Table 2. Behavioural recordings obtained from the chromatic stimuli, presented as disks of different diameters (4.6 or 8 cm). $B/L = B/F$ is the proportion of bees that effectively learned the training stimulus at the first distance (20 cm) from the total number of bees trained. $N$ is the number of bees tested in each case. $x_{det}$ is the minimum visual angle at which detection is still possible; $x_{indet}$ is the maximum visual angle at which detection disappears; both are given with their lower and upper limits, calculated from the distances $L1$ and $L2$ (see Fig. 1B). $x_{min}$ is the limiting angle at which the bees detect a stimulus with a probability $P_d = 0.6$ is given by $x_{det} + x_{indet}/2$.

<table>
<thead>
<tr>
<th>Colour</th>
<th>$B/L / B/F$</th>
<th>Diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Uv-blue (HKS-33N)</td>
<td>8.11</td>
<td>$N = 3$</td>
</tr>
<tr>
<td>Blue 1 (HKS-41N)</td>
<td>6.6</td>
<td>$N = 2$</td>
</tr>
<tr>
<td>Blue 2 (HKS-33N)</td>
<td>8.8</td>
<td>$N = 4$</td>
</tr>
<tr>
<td>Green (HKS-3N)</td>
<td>12/12</td>
<td>$N = 4$</td>
</tr>
<tr>
<td>Uv-green 1 (HKS-8N)</td>
<td>8/8</td>
<td>$N = 4$</td>
</tr>
<tr>
<td>Uv-green 2 (HKS-82N)</td>
<td>9/11</td>
<td>$N = 5$</td>
</tr>
</tbody>
</table>

We further consider only the results obtained with the bee-chromatic stimuli (Table 2). The distances at which the bees were able to detect a particular disk and at which they could not detect it were concordant for all the bees tested. Thus, inter-individual variation was negligible in this task. Moreover, $x_{det}$, $x_{indet}$ and thus $x_{min}$ did not depend on the disk diameter (Table 2), showing that detection can be described entirely in terms of the visual angles subtended by the stimuli.

The range defined by the lower and upper limits of the visual angle decreases with an increase in the diameter of the disk; therefore, we used the results from the disk of the bigger diameter (8 cm) to estimate $x_{min}$ more precisely (see Table 2). The $x_{min}$ was the same (5°) for
the blue (blue 1 and blue 2), the green and one of the uv-green stimuli (uv-green 1), but clearly differed from the $x_{\text{min}}$ of the uv-blue and the other uv-green (uv-green 2) stimuli (15°).

We defined $x_{\text{min}}$ as the subtended angle at which the bees detect a given stimulus with a probability $P_0 = 0.6$ (see Materials and methods). However, $x_{\text{min}}$ should depend on the established level of probability of detection $P_0$. In order to find out how the probability of detection varies with the visual angle, we plotted the percentage of correct choices as a function of the visual angle. Because the behaviour of the different bees was consistent and depended only on the visual angle and not on the disk diameter, we pooled the data of individual bees for all diameters (Fig. 3). For all bee-chromatic stimuli, the probability of detection changes abruptly when $x$ is close to $x_{\text{min}}$. Thus, the value of $x_{\text{min}}$ is nearly independent of $P_0$.

Summing up, the results thus show that bees learn to detect the bee-chromatic colours (uv-blue, blue, green, and uv-green) easily but not the achromatic one (human pink). Moreover, $x_{\text{min}}$, the visual angle at which the bees detect a given stimulus with a probability $P_0 = 0.6$, does not depend on the absolute disk diameter but only on the angle subtended by the disk at the bee's eye. The $x_{\text{min}}$ was the same for the blue 1, blue 2, green and uv-green 1 stimuli, namely, 5°, but clearly differed for the uv-blue and the uv-green 2 stimuli (15°).

**Discussion**

The experimental design applied in our experiments requires that the bee learns to associate a coloured cardboard disk with reward. Only then is it possible to establish whether the bee can detect the stimulus from a distance or not. This arrangement stimulates the detection of flowers by experienced foragers under natural conditions, because flower visiting is guided by appetitive associative learning, which then leads the bees to flowers according to the stimuli previously associated with food (Menzel 1985; Menzel et al. 1993).

Colour stimuli are learned by bees particularly effectively (Menzel 1967), and are later used to detect and recognize similar potential food sources. An achromatic stimulus, like the human pink used in our study, may, however, be learned only weakly. This finding is in agreement with Daumer's observation (1956, p. 449–450) that the training of bees to a bee-white (i.e. an achromatic stimulus) against a blue-green stimulus is not as efficient as the reciprocal training, and with the findings by Backhaus et al. (1987) showing that colour vision during close-up discrimination may not include a perceptual dimension for colour brightness. An achromatic stimulus may be, however, learned if its overall intensity contrast against the background is sufficiently high. For instance, bees presented with black disks on a white background (87% overall intensity contrast) in the study of Lehrer and Bischof (1995), detected them very well according to our 60% criterion. However, at least thirty learning trials were necessary for the bees to achieve this task. This strongly contrasts with the three learning trials necessary to consolidate permanently a colour in honeybees' memory (Menzel 1968). Recently, Lehrer and Bischof (1995) have demonstrated that the performance of bees trained to black and grey disks on a white background depends on the overall intensity contrast of the stimuli. In their experiments, a black disk with 87% overall intensity contrast yielded a choice frequency of 74% when it subtends an angle of 30° (the angle subtended by our 8 cm disk at the control distance), whereas grey disks with overall intensity contrasts of 68% and 29% yielded choice frequencies of only 65% and 56%, respectively (± 60%, considering the confidence intervals that may be calculated). Thus, an overall intensity contrast of 41.6%, like that provided by our pink achromatic stimulus, may not have been sufficient to allow reliable learning (choice frequency > 60%), even after 50 learning trials.

The role of spectral parameters in the detection of coloured stimuli

Different $x_{\text{min}}$ values were found for the six chromatic colours (Table 2). Blue (blue 1 and blue 2), green and uv-green 1 disks were best detected ($x_{\text{min}} = 5°$), whereas the uv-blue and the uv-green 2 disks were poorly detected ($x_{\text{min}} = 15°$). The best detected blue 1, blue 2, green and uv-green 1 stimuli have different chromatic contrasts (4.6, 5, 8.3 and 5.4 COC units respectively; see Table 1); however, the uv-blue and the uv-green
2 stimuli have nearly the same chromatic difference from the background as that of the blue 1 stimulus (about 4 COC units). Thus, the differences in detectability of chromatic stimuli cannot be explained in terms of their chromatic contrast. We analysed, therefore, our stimuli with respect to the contrast they produce in individual receptor types in order to test whether receptor-specific or overall intensity contrasts contribute to the difference between these two groups of stimuli.

The green stimulus offered a low overall intensity contrast to the background (4.6%), and the blue 2 stimulus a five times higher contrast (25%) (Table 1). The performance of the bees was, nevertheless, the same (Table 2). In the same way, the fact that both stimuli that were poorly detected, uv-blue and uv-green 2, had a magnitude of intensity contrast comparable to that of the best detected stimulus blue 2 (25%) allows us to conclude that the magnitude of overall intensity contrast is not a relevant parameter in the detection task. This agrees with the results of Lehner and Bischof (1995), who also showed that chromatic stimuli with different overall intensity contrasts (2% and 36%) are detected at the same visual angle. Nor is the sign (i.e. decrease or increase in relation to the background) of the overall intensity contrast the critical parameter: blue 1 and blue 2 disks had comparable magnitudes but opposite signs of overall intensity contrast (−16% and 25% respectively; see Table 1) and resulted, nevertheless, in the same behavioural performance. This conclusion is supported by the values of the overall intensity contrast of the two other stimuli that were poorly detected: uv-blue and uv-green 2 disks presented overall intensity contrasts of 27.4% and −20% respectively, and resulted in the same vision (15°). This shows that the sign of the overall intensity contrast is not relevant for the detection task. It may be argued that, in pattern discrimination, black figures on a white background are much more effective than white figures on a black background (von Weiszäcker 1970; Wehner 1972, 1973), and thus the sign of intensity contrast may play a role. However, the bees’ task in our experiments was not pattern discrimination, but rather colour learning and contrast-dependent detection. Thus, what may be valid for pattern learning and discrimination may not apply in our detection context. As for colour discrimination experiments (Daumer 1956; Menzel 1967; von Helversen 1972; Backhaus et al. 1987; Backhaus 1991; Brandt et al. 1993; Chittka et al. 1992), colour detection and recognition appear to be independent of intensity contrast. We need, therefore, to concentrate on the receptor specific contrasts in order to explain these results.

The blue 1 and uv-green 1 stimuli allowed us to check specifically for the effects of contrast to the blue and uv receptors respectively, because they did not present such contrasts (blue 1: 0.4% of blue contrast; uv-green 1: −0.7% of uv contrast; see Table 1). Because they resulted in the same performance (vision 15°), it may be concluded that neither ultraviolet nor blue-receptor contrast are relevant in the detection task. This conclusion is valid not only for the amount but also for the sign of such specific receptor contrasts: blue 2 and green disks had comparable amounts but opposite sign of uv contrast (12% and −10.2% respectively) and of blue contrast (48% and −36.5% respectively) and were, nevertheless, equally detectable (vision 15°). By comparing the best detected stimuli (vision 5°) with the poorly detected ones (vision 15°), it is obvious that differences in vision are not correlated with differences in single ultraviolet and/or blue contrasts.

The poorly detected uv-blue and uv-green 2 disks allowed us to check specifically for the effects of contrast to the green receptor because these stimuli did not present such a contrast (−0.1% and 0.1% of green contrast respectively; see Table 1), whereas all the best detected stimuli did. In this case, abolition of the green contrast changed the behavioural performance drastically. Even if both uv-blue and uv-green 2 were clearly different in their hue (Fig. 2b), they resulted in the same impairment of the detection performance (vision 15°). Together with the fact that the other spectral characteristics do not account for the differences in detectability, this allows us to conclude that green-receptor contrast is involved in the far-distance detection performance: when it is absent, detection is impaired. However, blue 2, uv-green 1 and green stimuli were detected at the same vision (15°) and had, nevertheless, different magnitudes of green receptor contrast (14.0%, 21.6% and 60.6% respectively; see Table 1). This indicates that for far-distance detection the contrast to the green receptor should be above a critical threshold which lies between 0% and 14%. Further increase in green contrast does not lead to an increase in the distance from which an object can be detected. This conclusion is confirmed by the results of Lehner and Bischof (1995) who found the same detection performance with 2 chromatic disks that produced different amounts of green contrast against the background (63% and 20%). The authors concluded that green contrast is not likely to be involved in the detection task. According to our results, it seems more probable that green contrast is actually involved in the far-distance detection task, and that Lehner and Bischof were not able to find a significant effect of this parameter because their stimuli were above the critical threshold for contrast to the green receptor type. Contrast to the green receptor also mediates other visually guided tasks involving the use of image motion (see review in Lehner 1987, 1993). Interestingly, the optomotor response of tethered bees, flying within a vertically striped drum which rotates around them, is mediated by the green receptor and requires a minimal contrast of 7.2% (Kaiser and Liske 1974), a value which supports our conclusions.

It thus appears that the presence of chromatic as well as green contrast is necessary for learning a colour
target and for detecting it at further distances ($\phi_{\text{min}} = 5^\circ$). Values of 3.6° and 5.3° for $\phi_{\text{min}}$ have been reported by Lehrer and Bischof (1995) for their two combinations of chromatic stimuli. These are close to the one we found for the stimuli that are detected best (5°), especially taking into account the lack of confidence intervals for the choice percentages reported in their work and the difference in the definition of $\phi_{\text{min}}$, resulting from their different experimental set-up.

The number of ommatidia involved in the detection of coloured stimuli

Detection can be considered as a threshold phenomenon: a stimulus is detected only when a certain minimal number of ommatidia receive a sufficient signal. In principle, an object having sufficient contrast to the background might be detected even if it projects onto an area inferior to that of one ommatidium, providing that the contrast is high enough to create a response different from that in the surrounding ommatidia. It has been shown, for example, that honeybee drones are able to detect a moving object (dummy queen) projecting onto an area which is less than that of one ommatidium (Vallet and Coles 1993). However, because the target moved in this experiment, it is not obvious that only one ommatidium was involved in the detection task. Lehrer and Bischof (1995) concluded that stimulation of only two ommatidia in the vertical direction, and of only one ommatidium in the horizontal direction, is sufficient for detection. However, the authors did not take the overlap of the visual fields of adjacent ommatidia into account, i.e. the fact that ommatidia covered by a dark stimulus also receive the signal from a bright background, and vice versa. Thus, both the interommatidial angles ($\Delta \phi$) and the acceptance angles ($\Delta \rho$) should be taken into account.

The ommatidia form an hexagonal lattice (Braitenberg 1970), which is characterised by the primitive translation vectors in the horizontal ($a_0$) and vertical directions ($a_0$). The orientation of the visual axes of the ommatidia can be calculated from the interommatidial angles in the horizontal ($\Delta \phi_h$) and the vertical ($\Delta \phi_v$) planes (Stavenga 1979). Kirschfeld (1973) and Seidl (1980) showed that in the frontal region of the bee eye, $\Delta \phi_h = 1.6^\circ$ and $\Delta \phi_v = 0.9^\circ$. Because the eye of the honeybee is ovaly shaped, the projection of a disk like those used in our experiments onto its frontal region results in the vertical oval presented in Fig. 4. Therefore, the number of ommatidia covered by the circular target in the vertical direction is greater than that in the horizontal direction.

Because the visual fields of adjacent ommatidia overlap, the ommatidia that are covered by the stimulus also receive the signal from the background, and those adjacent that look at the background also receive the signal from the stimulus. Excitation (E) produced by a given object is calculated by integration of the angular sensitivity function, $A(\Phi_h, \Phi_v)$, over the area (S) of the object (in angular measure):

$$ E = \frac{1}{S} \int_0^S A(\Phi_h, \Phi_v) d\Phi_h d\Phi_v $$

where $\Phi_h$ and $\Phi_v$ are the angular coordinates in the horizontal and vertical directions respectively, and C the proportionality factor which depends on the spectral properties of the object. The angular sensitivity function can be approximated by the Gaussian function (Götz 1964; Tunstall and Horridge 1967; Snyder 1979):

$$ A(\Phi) = \exp[-2.77(\Phi/\Delta \rho)^2] $$

where $\Delta \rho = 2.6 \pm 0.7^\circ$ (Laughlin and Horridge 1971).

Excitations of ommatidia produced by the circular stimuli we used were calculated with Eqs. 1 and 2. Because the blue 1, blue 2, green and uv-green 1 stimuli yielded the same $\phi_{\text{min}}$ (5°) but had different green contrast, it may be concluded that the relative, rather than
the absolute excitation values are relevant. To calculate these values, the excitation values we obtained were compared with those of the ommatidium looking to the centre of the stimuli. Three categories of relative excitations are depicted in Fig. 4. The six-pointed symbol indicates the ommatidia with excitations higher than 75% of that of the central one; the three-pointed symbol those with excitations between 50% and 75%, and the circle those with excitations between 25% and 50%; the ommatidia with excitations lower than 25% are not indicated.

In the case of the $x_{\min}$ subtended by the targets with green contrast (5°), the ommatidia covered by the stimulus are excited to values of approximately 70% of those obtained in the central ommatidium. The ommatidia that are not covered are excited to values lower than 25%. In the case of the $x_{\min}$ subtended by the targets without green contrast (15°), nearly all ommatidia covered by the stimulus are excited to values higher than 75%, and several neighbouring ommatidia are excited to values higher than 50% (Fig. 4). Thus, the number of ommatidia involved in the detection of a given stimulus depends not only on the direction (vertical or horizontal) in which the ommatidia are counted, but also on the relative magnitude of excitation which is considered to be important for detection.

If we assume that all ommatidia with relative excitation values higher than 50% are important for the detection task, the total number of ommatidia allowing effective detection is equal to 7 for the stimuli with chromatic and green contrast ($x_{\min} = 5°$), and 59 for the stimuli providing only chromatic but no green contrast ($x_{\min} = 15°$). Thus, the number of critical ommatidia is much larger for the stimulus without green contrast than for the stimuli with green contrast.

Chromatic and green contrast have different relevance at different distances

Finally, we draw special attention to the striking phenomenon that, in this experimental context, bees appear to be unable to learn targets if they contain only green contrast, but no chromatic contrast, as is evident from the finding that the achromatic stimulus was not detected despite the fact that it produced 41% green contrast against the background (see Table 1), whereas the stimuli that were detected best ($x_{\min} = 5°$) were those that produced green contrast as well as chromatic contrast against the background. These results suggest that 1) stimuli have different behavioural relevance at different distances and that 2) green contrast may be learned through the presence of chromatic contrast in the training signal.

To explain the phenomena observed, we propose that, in the context of our study, the excitation difference in the green photoreceptor, between target and background (i.e. green contrast), is always perceived prior to colour during an approach flight. This is demonstrated by the fact that absence of green contrast in some of our targets leads to shorter detection ranges (i.e. larger $x_{\min}$). Both the green and the chromatic contrast signals would appear at each approach flight in a regular temporal order: first, green contrast, then, chromaticity.

Green contrast may be learned through the presence of chromatic contrast

We still need to explain why the signal mediated through the green receptors is not learnt to be used as the only cue in the detection task. Using exclusively green contrast, bees may learn to detect edges (Lehrer et al. 1990), discriminate objects from the background (Lehrer and Srinivasan 1993) and estimate their distance from appetitive signals (Lehrer et al. 1988). However, what is learned in these situations is not the green contrast per se, but rather the motion cues of the stimulus, which are analysed through a particular perceptual channel, the one associated with the green receptors. In our experimental context, the green signal is not related to evident motion cues and thus would have a low salience leading to no or little learning. Together with a colour cue, however, it is incorporated into the detection task.

Considering our results and explanations in the framework of the modern theories of learning, the situation we describe resembles that of a facilitation process, in which learning of a conditioned stimulus (CS) is facilitated by another stimulus provided in compound with the CS (Holland 1983; Rescorla et al. 1985; Domjan and Burkhard 1986). In our case, it is possible that chromatic contrast facilitates in some way learning of the green-contrast signal, thus enlarging the bees' spatial detection range. Our hypotheses lend themselves to critical testing because the temporal sequence, the combinations of green and colour signals, and their relationships to the reward can be varied systematically.

Ecological and behavioural implications

If the hypotheses outlined above apply, they are highly relevant in an ecological context. Objects with green contrast that makes them detectable at a far distance, would in principle be neglected as food signals in the natural environment; however, such objects become a potential far-distance flower cue if they were learned together with the floral chromatic contrast. Thus, using green and chromatic contrast a bee searching for a particular flower signal would turn towards a potentially interesting object at treble the distance than when relying solely on chromatic contrast. Flowers provide a wide range of green contrasts together with their chromatic contrast (Menzel and Shmida 1993). The
relationship between colour and green signal in flowers needs to be analyzed now in detail to examine whether there is any systematic or constraint combination of these two components of the visual signals from flowers. Our results also imply that the traditional concept of flowers being “long-distance signals” for pollinators like the honeybee (von Frisch 1919, 1965, p 521; Kevan and Baker 1983) needs to be reconsidered. Corollas of most species have diameters of less than 5 cm and, in the best case (\( \alpha_{\text{min}} = 5^\circ \)), the farthest distance from which these corollas would be detectable is about 45 cm. Visual detection may occur at even shorter distances where odour may also play a role (Butler 1951; Kevan 1989). On the other hand, the farthest distance from which a “big” corolla (12 cm) would be detectable is about 135 cm in the best case (\( \alpha_{\text{min}} = 5^\circ \)). The corporate stimuli of inflorescences or groupings of flowers (patches etc.) explain their greater rates of visitation by pollinators vis a vis individual flowers, small inflorescences, or small groupings (see Kevan et al. 1990). However, in such groupings the flowers are interspersed with vegetation and colour mixing effects are expected to influence attraction. How these effects influence detection remains to be solved.

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