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Colour preferences of flower-naive honeybees

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Abstract Flower-naive honeybees *Apis mellifera* L. flying in an enclosure were tested for their colour preferences. Bees were rewarded once on an achromatic (grey, aluminium or hardboard), or on a chromatic (ultraviolet) disk. Since naive bees never alighted on colour stimuli alone, a scent was given in combination with colour. Their landings on twelve colour stimuli were recorded. Results after one reward (“first test”) were analysed separately from those obtained after few rewards (“late tests”).

- 1) After pre-training to achromatic signals, bees preferred, in the first test, bee-uv-blue and bee-green colours. With increasing experience, the original preference pattern persisted but the choice of bee-blue and bee-green colours increased.
- 2) Neither colour distance of the test stimuli to the background or to the pre-training signal, nor their intensity, nor their green contrast, accounted for the colour choice of bees. Choices reflected innate preferences and were only associated with stimulus hue.
- 3) Bees learned very quickly the pre-trained chromatic stimulus, the original colour preferences being thus erased.
- 4) Colour preferences were strongly correlated with flower colour and its associated nectar reward, as measured in 154 flower species.
- 5) Colour preferences also resemble the wavelength dependence of colour learning demonstrated in experienced bees.

Key words Honeybees · *Apis mellifera* · Colour vision · Colour preferences · Flower colours

Introduction

“Bees and various other insects must be directed by instinct to search flowers for nectar and pollen, as they act in this manner without instruction as soon as they emerge from the pupa state”. In this way, Charles Darwin (1877, Chap. XI, p. 425) anticipated, more than a hundred years ago, the idea of innate predispositions which could allow a pollinator to select a potential food source prior to any experience and could also facilitate the choice behaviour after learning. However, the existence of preparedness for certain sensory cues, in particular for visual cues, is very difficult to demonstrate since the experience and motivation of the individual forager to be tested must be carefully controlled (Menzel 1985).

The honeybee *Apis mellifera* has been, through the years, the subject of several studies that attempted to tackle the question of innate colour preferences (Oettingen-Spielberg 1949; Butler 1951; Ludwig -cited in von Frisch 1967-; Banschbach 1994). However, the answers provided are questionable due to the lack of appropriate control of the bees' previous experience and motivation in the test situation. An usual procedure was to train bees to collect sucrose solution at a site that was not associated with any particular colour, and then give them, at the same site, a choice among various colours that were all new to the bees. However, a successful forager quickly learns any colour mark close to the nectar source (even seemingly colour neutral stimuli, such as grey disks) and directs its choice towards colour stimuli according to their perceptual similarity to the learned signal (Daumer 1956; Menzel 1967, 1968, 1985; Backhaus et al. 1987; Giurfa 1991; Chittka et al. 1992). Under these conditions, the resulting performance may have reflected learned, and then

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generalized, rather than innate predispositions, and the word "spontaneous" is meaningless.

Preparedness for learning particular colours as food signals by honeybees has been demonstrated by Menzel (1967). In this study, variation of the pre-training conditions, the stimuli properties, the alternative colours and the test situations suggested that the differences found in the bees' learning performance reflect a colour specific evaluation process independent of former experience, test conditions and colour similarities. However, since no naive bees were used in these learning studies, it should be concluded that colours are differentially valued as signals of expected food sources, only with respect to the learning process and not to the choice performance before learning a new colour signal.

To test innate colour preferences, flower-naive honeybee foragers must be used, their experience prior to tests has to be controlled and the motivation to search at a particular area should be induced with as little pre-training as possible. Taking this into account, we conducted experiments with flower-naive honeybee foragers whose experience and motivation were carefully controlled and asked whether these foragers showed spontaneous colour preferences. We investigated whether the bees' choices can be accounted for by spectral parameters of the stimuli used or rather reflect an innate weighting function which puts the colour stimuli in a particular rank order. Finally, we analyzed whether the colour choice of naive bees has a natural correlate in floral coloration and its associated nectar reward.

Materials and methods

Experiments were performed in a flight cage (3 × 5 m × 1.90 high) covered with a nylon net and kept under natural light and temperature conditions. A small hive with five frames was mounted in a corner of the enclosure. The hive was divided into two sections by a 2 mm wire mesh: one section, with three frames and containing the queen, had free flight access to natural food sources outside the flight cage. Pollen and nectar, necessary for sustaining the colony, were thus provided by its foragers. The other section, with two frames, opened to the cage; honeybees from this part neither flew outside nor had any experience of natural food sources. These flower-naive honeybees received no rewards on colour stimuli except those offered in the experiments and explicitly chosen to this end.

Frames with capped brood maintained at 34–35°C in an incubator, and obtained from donor colonies, provided the foragers used in the experiments. Emerged workers were marked with a coloured spot on the thorax according to their birthday and were introduced into the section of the hive that opened to the flight cage. A nucleus of approximately 1200 marked bees was obtained in this way. Experiments were performed between the tenth and fifteenth day after emergence.

Preliminary experiments were performed to analyse whether flower-naive honeybee foragers instinctively choose colour stimuli without the aid of additional cues. In no case did flower-naive bees alight on unscented coloured disks distributed within the flight cage. When these were presented, bees disregarded them and it was impossible to make them choose colour stimuli without previous training.

The training box

Therefore, bees were trained to a food source, maintained in the darkness of a training box and scented with Geraniol. The training box (15 × 15 × 20 cm), made of cardboard, was placed on the floor of the flight cage (Fig. 1a). The food source provided 50% w/w (weight/weight) sucrose solution ad libitum; the scent came from a vial with paraffin oil and Geraniol (10:1). The inside of the training box was painted black and a lateral entrance, protected by a light trap, allowed access to the food source. A training stimulus (in the form of a perforated disk, 7 cm diameter) was put on the entrance. The side of the box presenting the training stimulus was never exposed to direct sunlight.

The test box

A test box (50 × 50 × 20 cm) was made of cardboard and covered with an horizontal hardboard plate (75 cm diameter) presenting twelve holes (1 cm diameter) (Fig. 1b). These holes were 15 cm apart, were closed with a net so that bees could not enter the box. Disks of coloured cardboard or colour filters (7 cm diameter) (Schott, Mainz), placed on top of sanded aluminium plates (Lieke and Menzel 1983) were presented horizontally on the hardboard, directly above the holes. Inside the test-box, a Petri dish with paraffin oil and Geraniol (10:1) was placed so that odour evaporated evenly through the plate holes and the holes in the centre of the colour disks.

Stimuli

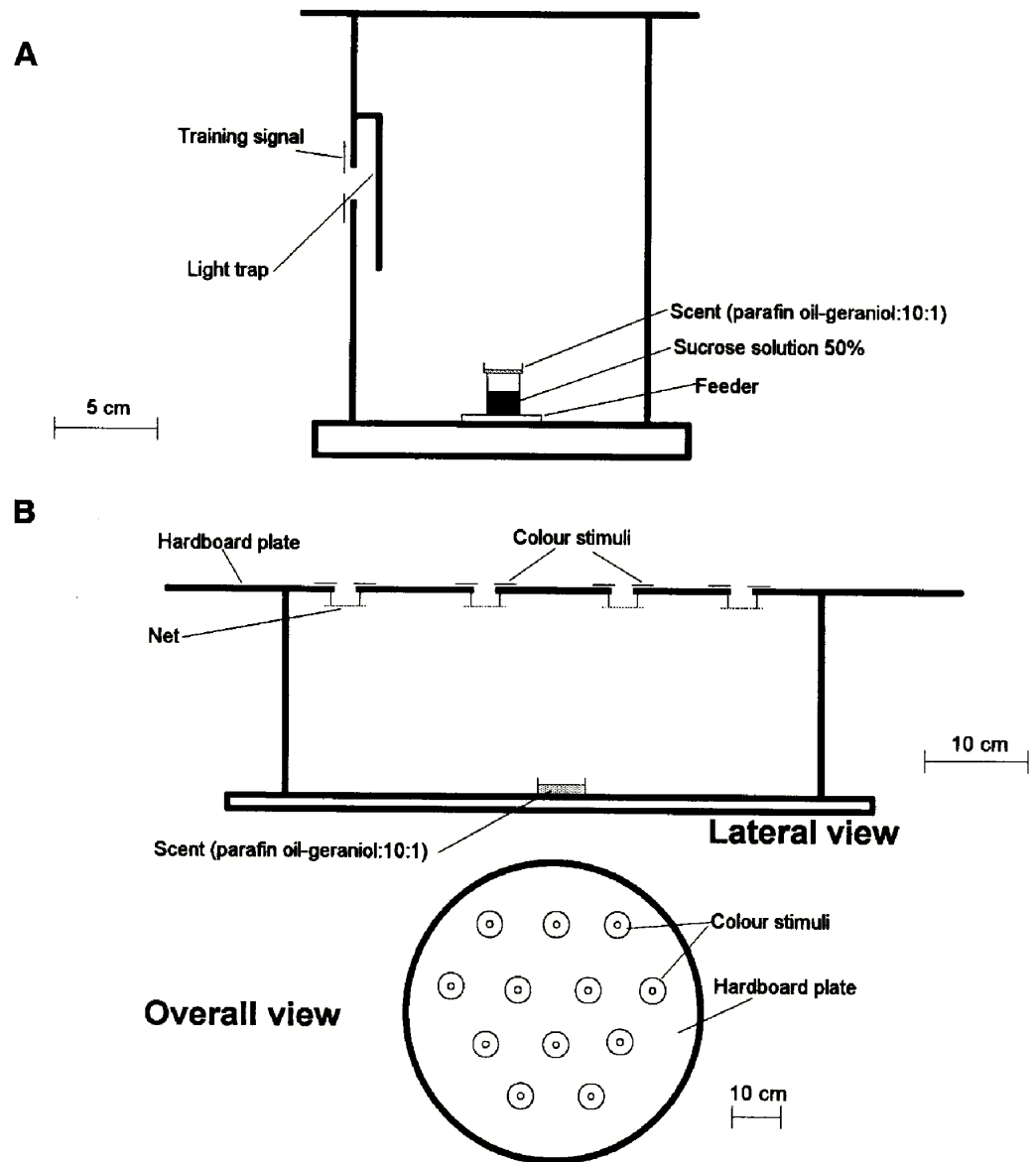
Training stimuli

During training, the entrance of the training-box presented a disk of one of the following types: sanded aluminium, a grey cardboard, the hardboard used for constructing the test-box plate or an ultraviolet colour filter (Schott filter UG1). The first three stimuli were chosen as "neutral" stimuli because they possess quite even, flat spectral reflectance curves throughout the spectral visual range of the honeybee. The fourth stimulus (UG1) was chosen as a clear chromatic signal to compare the effect of pre-training on it with that of pre-training to the "neutral" stimuli. By placing the training stimuli vertically, but the test stimuli horizontally, we attempted to make the situation associated with reward as different from the test situation as possible.

Test stimuli

During tests, twelve colour stimuli were presented (Table 1), thus providing a simultaneous multiple choice situation. From these twelve stimuli, three were coloured cardboards (B2, G1, Y2) and the rest were colour filters (UG1, UG3, BG18, BG24, BG25, BG28, VG6, GG495, OG550), each placed on top of a sanded aluminium disk. The spectral reflectance curves of the stimuli were measured using a flash spectrophotometer (SR01, Gröbel UV Elektronik GmbH, resolution 1 nm), with BaSO₄ as a standard reference. Figure 2 shows the loci of the stimuli in the colour hexagon of the honeybee (Chittka 1992), a colour space that plots the three photo-receptor excitations at an angle of 120° and represents excitation values for any type of spectrally opponent mechanism whose weighting factors add up to zero. The hexagon is a generalized colour opponent space whose metrics have been shown to be applicable to numerous species of bees and wasps (Chittka et al. 1992). The colour space allows an estimation of perceptual differences among colours, and accounts for a variety of phenomena associated with colour

Fig. 1 **A** The training box;
B The test box in lateral and overall views



vision in honeybees (Chittka 1992). The space is divided into six equally large areas (uv; uv-blue, blue, blue-green, green and uv-green) which refer to the way the bee receptors are differentially stimulated by a given spectral stimulus (Chittka et al. 1994). These categories have proven to be successful in characterizing floral colours in terms of honeybee colour vision (Menzel and Shmida 1993; Chittka et al. 1994) and will be used throughout this text.

Table 1 lists the test stimuli, and gives their perceptual distances to the hardboard background on which they were presented, their perceptual distances to the training signals, as well as their intensity values (measured as the sum of excitations of the three spectral receptor types after adaptation to the background), and their specific green contrast values. Perceptual distances between two colour loci in the colour hexagon are calculated by means of Euclidean metric (see Chittka 1992 for detail). 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 is widely accepted (Backhaus and Menzel 1987; Backhaus 1991; Menzel and Backhaus 1991) and has proved to hold, at least for the bee's

phototactic response (Menzel and Greggers 1985). Contrast to green receptor (Srinivasan and Lehrer 1984) was calculated as the excitation of the green receptor produced by a given colour stimulus, relative to that produced by the background. This particular contrast is relevant because it has been proved that green sensitive receptors mediate a series of visually guided tasks related to self-motion in bees (see review in Lehrer 1987, 1993). Since no similar effect of ultraviolet- and blue contrasts has been proven up to now, we concentrated specifically on the effect of green contrast. We also calculated the dominant wavelength for every stimulus (Wyszecki and Stiles 1982). This was done to assign a particular wavelength to our broad band spectral stimuli, using the colour hexagon as a graphical representation of colour loci. The dominant wavelength of a colour stimulus correlates with what is called, in reference to human colour vision, the stimulus hue and can be obtained through a graphic procedure. The intercept of a line traced from the centre of the colour diagram, passing through a particular stimulus, with the spectral curve determines the corresponding dominant wavelength on the spectral curve, which defines the stimulus hue (Table 1).

Table 1 Spectral properties of the training- and test stimuli used. Intensity is measured as the sum of excitations of the three spectral receptor types after adaptation to the background. Green contrast is measured as the specific excitation of the green receptor, relative to that of the background. *NP* means that the stimulus was not

| Stimuli: | Hard. | Alu | Grey | u1 | u-b1 | u-b2 | u-b3 | u-b4 | u-b5 | b1 | b-g1 | b-g2 | g1 | g2 | g3 |
|------------------------------------------|-------|------|------|-------|------|------|------|------|------|------|------|------|------|------|------|
| Distance to Background (Hexagon units) | 0 | 0.14 | 0.09 | 0.044 | 0.39 | 0.35 | 0.45 | 0.24 | 0.26 | 0.10 | 0.02 | 0.17 | 0.3 | 0.13 | 0.14 |
| Distance training signal (Hexagon units) | | | | | | | | | | | | | | | |
| Aluminium | NP | NP | NP | 0.033 | 0.25 | 0.21 | 0.31 | 0.11 | 0.15 | 0.12 | 0.16 | 0.30 | 0.45 | 0.26 | 0.27 |
| Grey | NP | NP | NP | 0.36 | 0.28 | 0.23 | 0.33 | 0.11 | 0.15 | 0.08 | 0.13 | 0.27 | 0.43 | 0.25 | 0.25 |
| Hardboard | NP | NP | NP | 0.44 | 0.39 | 0.35 | 0.45 | 0.24 | 0.26 | 0.10 | 0.02 | 0.17 | 0.30 | 0.13 | 0.14 |
| u1 | NP | NP | NP | 0 | 0.17 | 0.20 | 0.26 | 0.35 | 0.38 | 0.44 | 0.46 | 0.61 | 0.72 | 0.51 | 0.50 |
| Intensity | 1.50 | 2.08 | 2.21 | 1.03 | 1.30 | 1.67 | 1.45 | 1.15 | 1.83 | 1.19 | 1.55 | 1.19 | 0.74 | 0.81 | 1.89 |
| Green Contrast | 0.50 | 0.60 | 0.65 | 0.13 | 0.19 | 0.34 | 0.19 | 0.23 | 0.44 | 0.35 | 0.52 | 0.48 | 0.44 | 0.35 | 0.71 |
| Dominant wavelength (nm) | AL | 393 | 402 | 366 | 390 | 392 | 398 | 409 | 414 | 434 | 483 | 501 | 524 | 554 | 563 |

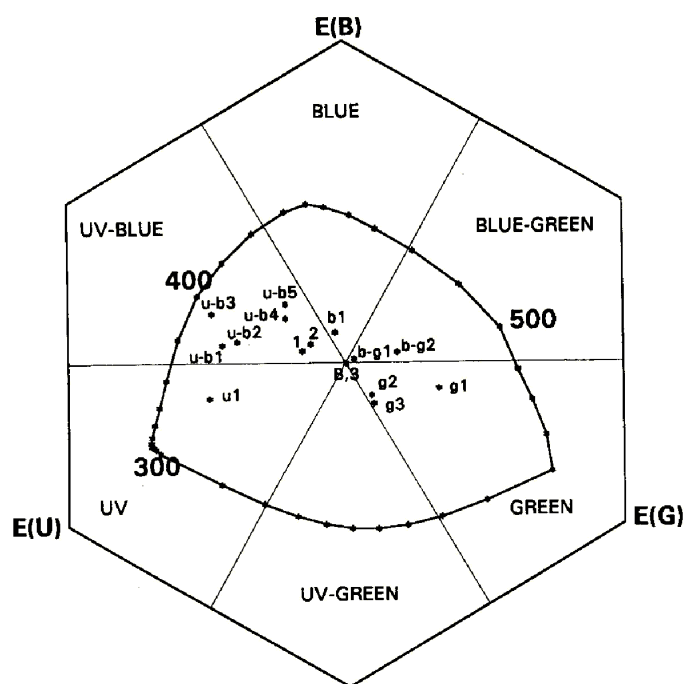


Fig. 2 Colour loci of the stimuli used, presented in the colour hexagon of the honeybee. Photoreceptor excitations at an angle of 120° and the spectral curve at adaptation light intensity are plotted. The loci along the spectral line are marked in 10 nm steps, and the mixtures of 300 and 550 nm (UV-green) in 10% steps. The hexagon is divided into six equally large areas (UV, UV-BLUE, BLUE, BLUE-GREEN, GREEN, UV-GREEN) which refer to the way the bee receptors are differentially stimulated by a given spectral stimulus. The middle point (*B*) represents the adaptation light (hardboard background); 1: Aluminium disk; 2: Grey disk; 3: Hardboard disk; *u1*: UG1 (F); *u-b1*: BG24 (F); *u-b2*: UG3 (F); *u-b3*: BG25 (F); *u-b4*: BG28 (F); *u-b5*: B2 (C); *b1*: BG18 (F); *b-g1*: G1 (C); *b-g2*: VG6 (F); *g1*: GG495 (F); *g2*: OG554 (F); *g3*: Y2 (C). (F) : Schott filter; (C): Cardboard

presented during tests, during tests, after training to a particular signal. *AL* means adaptation light; since it represents the centre of the colour hexagon, no dominant wavelength may be assigned in this case (see Materials and methods for more detail)

Procedure

Marked bees were trained to enter the training-box through the lateral hole to find the sucrose solution therein. To this end, marked bees were fed on a dish with diluted sucrose solution (20%) scented with Geraniol, placed at the hive entrance. The dish with the bees was then carried from the hive entrance and placed in the training box, where the bees found the 50% sucrose solution scented with Geraniol.

Bees were displaced in that way once or twice. They left the training box through the lateral hole and later returned actively. A foraging swarm (three to four marked bees foraging "in phase") was established by caging the other foragers for later use. After the first active visit, the training box was covered with a glass dome. Returning foragers thus only had access to the test box and a first test was performed. It was expected that bees of the foraging group would search among the plate holes for the previously learned mixture of paraffin oil and Geraniol.

Landings on a particular colour were recorded by observation. Results presented correspond to the choices of the group of foragers. After the first test, the training box was again presented, the bees were rewarded once and tested again as explained above. The position of the stimuli on the test box was randomised between test visits and fresh stimuli were always used to avoid the influence of foraging pheromones. Each test lasted for 3 min.

Flower colour reliability

To see whether potential colour preferences investigated here might be related to some correlation between flower colours and nectar rewards in the natural world, we chose a nature reserve near Berlin as our study site (Naturschutzgebiet Lange Dammwiesen, Strausberg, Brandenburg, Germany). Five sites were selected within this area, and we measured the spectral reflectance properties of all flower species that bloomed there over the year. In this fashion, we quantified the bee-subjective colour properties of 154 flower species (for details see Chittka 1992; Chittka and Menzel 1992). We then asked whether certain colours advertise a high nectar reward with

a higher reliability than others. For this purpose, the 154 flower species were first subdivided into the six bee colour categories (see above). Next, the flower species within each category were again separated into two groups: "high nectar flowers" and "low nectar flowers", according to the literature (Düll and Kutzelnigg 1994; Pritsch 1985). The flowers were then evaluated in terms of a quantity called "flower colour reliability". This quantity was calculated for each colour category by dividing the number of "high nectar flowers" within this category by the total number of species within this colour category, and multiplying the result by 100. Thus, if there are 30 species in the blue category, 15 of which are high-reward flowers, the flower colour reliability of "blue" is 50%. These data and concepts are an excerpt from a comprehensive study on the informational content of flower colours by Chittka, Kunze and Gumbert (unpublished; see also Chittka et al. 1993).

Statistics

The distribution of bee choices on the twelve test stimuli was analysed by means of the log likelihood ratio test (G statistic; see Zar 1985; pp 52–53) for goodness of fit, testing the null hypothesis of a random distribution of landings. The same procedure was applied to test the null hypothesis that colour choices are independent of the pre-training stimulus (Zar 1985; pp 71–72). To analyse whether colour distance to the background, to the pre-training signal, intensity or specific contrast to the green receptor type account for the bee's choices, the Spearman's rank correlation procedure was used (r_s ; Spearman rank correlation coefficient; see Zar 1985; pp 318–320). We used the same procedure to test the correlation between the naive-bee responses, the response of experienced bees in colour learning experiments (Menzel 1967), and flower colour reliability.

Results

Results were analysed distinguishing the landings recorded in a *first test* after only one pre-training visit with a "neutral" or a chromatic signal and the landings recorded after three to ten consecutive pre-training visits with a "neutral" or a chromatic signal (henceforth *late tests*). The former represent the colour choice of flower-naive bees when the pre-training experience was minimal and thus may depend more strongly on innate colour preferences of honeybee foragers. The latter involve the effect of cumulative experience. A comparison between both the first and late tests thus reveals whether or not the results of the first test indeed reflect spontaneous preferences.

In both cases, we asked whether naive foragers show colour preferences and tested whether the results obtained could be accounted for by 1) perceptual colour distance of the test stimuli to the background, 2) perceptual colour distance of the test stimuli to the pre-training signal, 3) some hypothetical intensity measure or 4) specific contrast to the green photoreceptor type (Srinivasan and Lehrer 1984). The first parameter may be related to the detectability of a colour signal since it represents its contrast to the background. Bees, for example, may choose the stimuli that are more detectable against a background, i.e. the stimuli with a greater perceptual distance to the background

(Lunau 1990; Chittka and Lunau 1992). The second parameter relates to the recognition of a colour signal, since it allows an analysis of whether bees choose colour stimuli on the basis of their similarity to the pre-trained stimulus. To this end, different pre-training colours were used. The third parameter was introduced to analyse whether bees choices relate to stimuli intensity, measured as the sum of excitations of the three spectral receptor types after adaptation to the background. Although an intensity coding channel is not necessary to explain the behaviour of experienced bees in colour discrimination tasks (Daumer 1956; Menzel 1967; Backhaus et al. 1987; Backhaus 1991, 1992; Brandt et al. 1993; Giurfa 1991; Chittka et al. 1992), naive bees may rely on this parameter to determine their first colour choices. Finally, the fourth parameter was incorporated to analyse whether bees' choices relate to the specific contrast to the green receptor. It is worth noting that perception of "green" (as a colour) has nothing to do with "green contrast". "Green" is a result of a particular excitation profile of all three spectral types of receptors, while "green contrast" involves the exclusive excitation of the green receptor in response to two different (adjacent) stimuli.

Pre-training to the achromatic stimuli

First test

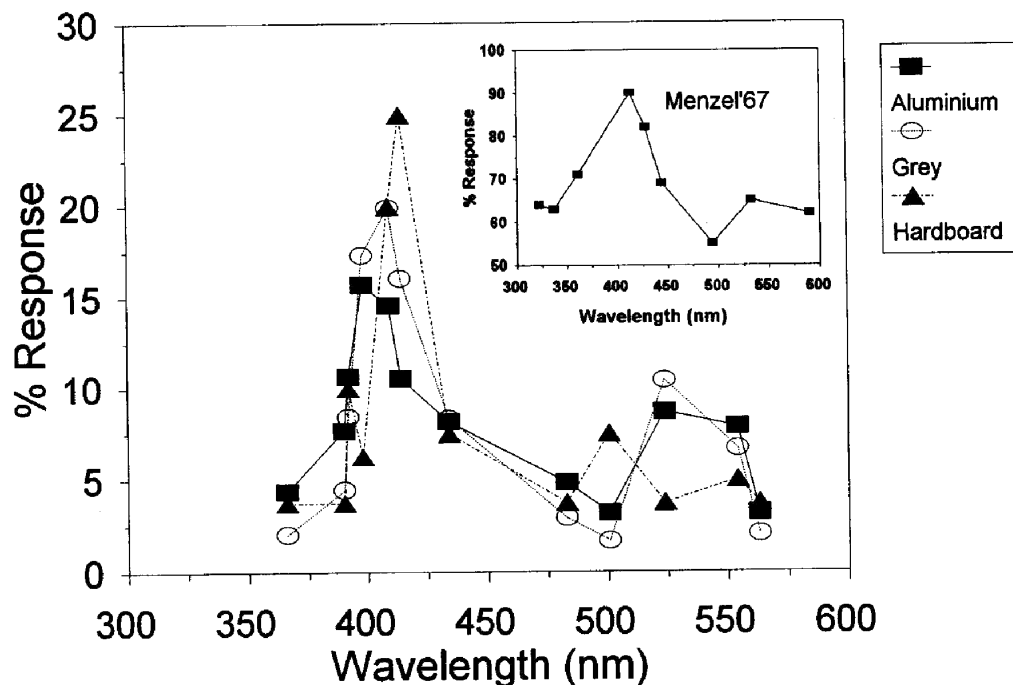
Bees trained to an aluminium disk (Fig. 3, squares) mostly prefer colour stimuli in the bee uv-blue category (around 410 nm) and in the green category (around 530 nm). The null hypothesis of a random distribution of landings on all colour stimuli must be rejected (281 landings; log likelihood ratio test: $G = 66.68$; $df: 11$; $P < 0.001$).

Bees trained to a grey disk (Fig. 3, empty circles) also prefer colour stimuli in the bee uv-blue and green categories (around 415 nm and 530 nm respectively). The distribution of the percentage of landings significantly deviates from a random distribution (538 landings; log likelihood ratio test: $G = 311.89$; $df: 11$; $P < 0.001$).

Bees trained to a hardboard disk (Fig. 3, triangles) clearly prefer colour stimuli in the bee uv-blue category (around 415 nm). The distribution of the percentage of landings significantly differs from a random distribution (80 landings; log likelihood ratio test: $G = 41.43$; $df: 11$; $P < 0.001$).

By means of the Spearman's rank correlation procedure, we tried to establish whether the choice frequencies of the test stimuli shown in Fig. 3 were significantly associated with the variation in the spectral parameters presented in Table 1. We find that neither perceptual distance to the test background (aluminium disk: $r_s = -0.39$; $n = 12$; NS; grey disk: $r_s = 0.30$, $n = 12$, NS; hardboard disk: $r_s = -0.03$; $n = 12$; NS),

Fig. 3 Pretraining to achromatic stimuli. "First test": percentage of landings on the test stimuli after one reward on an achromatic signal (aluminium disk; grey disk; hardboard disk). *Inset:* wavelength dependence obtained in learning experiments with experienced foragers (from Menzel 1967: Figure 9)



nor similarity to the pre-training signal (aluminium disk: $r_s = -0.31$, $n = 12$; NS; grey disk: $r_s = -0.20$, $n = 12$; NS; hardboard disk: $r_s = -0.03$; $n = 12$; NS), nor stimuli intensity (aluminium disk: $r_s = -0.002$, $n = 12$; NS; grey disk: $r_s = -0.08$, $n = 12$; NS; hardboard disk: $r_s = 0.19$; $n = 12$; NS), nor green contrast (aluminium disk: $r_s = -0.46$, $n = 12$; NS; grey disk: $r_s = -0.39$, $n = 12$; NS; hardboard disk: $r_s = -0.09$; $n = 12$; NS) can account for the naive-bees' choice after a single reward on achromatic signals. In other words, colour choice by naive bees is not determined by any of the above visual parameters but seems rather to reflect an innate weighting function which puts the colour stimuli in a particular rank order.

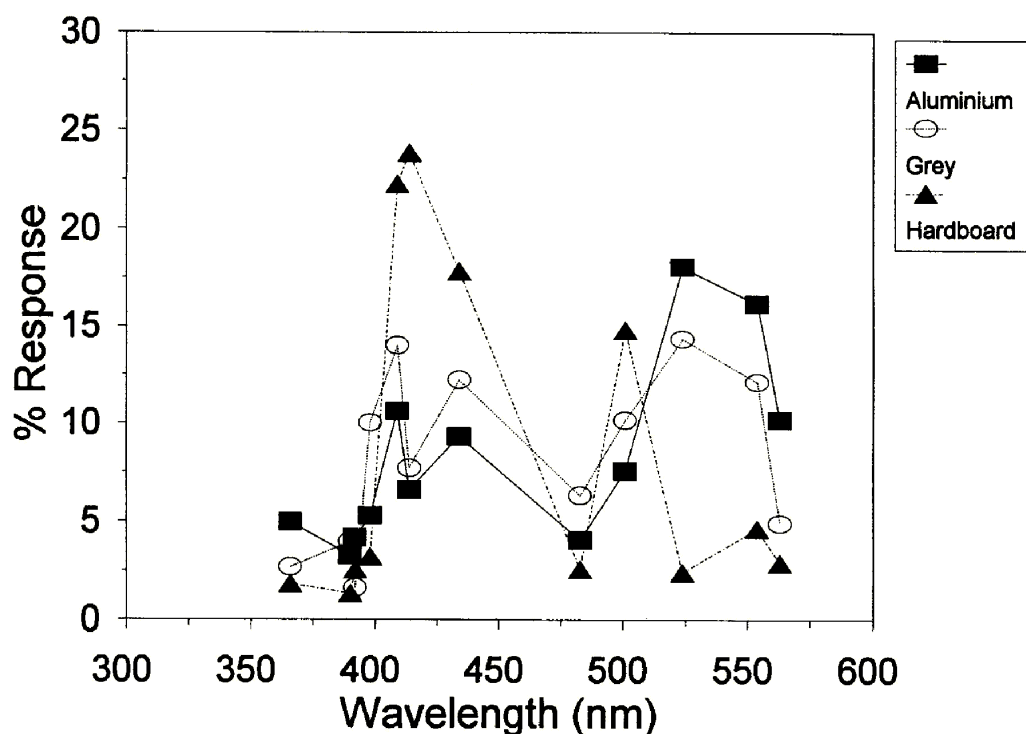
We tested the null hypothesis that colour choice is independent of the pre-training stimulus by means of log likelihood ratio tests, and found that all three distributions shown in Fig. 3 differ significantly from one another (aluminium vs grey: $G = 24.09$; $df: 11$; $P < 0.025$; aluminium vs hardboard: $G = 21.42$; $df: 11$; $P < 0.05$; grey vs hardboard: $G = 24.79$; $df: 11$; $P < 0.01$). We conclude that colour choice is not independent of pre-training stimulus and that even neutral signals may influence colour choice of naive-honeybee foragers. However, the nature of this influence is rather contra-intuitive. Stimuli with smaller colour differences to the pre-training signal would be expected to be chosen more frequently than such with large colour differences. The opposite was found. The pre-training stimuli aluminium and grey are shifted somewhat to the uv-blue corner of the colour space (Fig. 2). In this situation, uv-blue colours are chosen less frequently than when the hardboard disk is used as pre-training

signal, while the stimuli whose loci are in the opposite direction of the colour space (the green colours) are chosen more frequently. The pre-training stimulus Hardboard is closer to the green test stimuli than are the two other pre-training signals aluminium and grey. In this case, green test stimuli are hardly selected by the bees, while those on the opposite side of the colour space, the uv-blue colours, are chosen more frequently. This is in strong contrast to the similarity-based judgements of experienced workers (Daumer 1956; Menzel 1967; von Helversen 1972; Backhaus et al. 1987; Giurfa 1991). Since neither perceptual distance of the colour stimuli to the background or to the pre-trained signals, nor the intensity of the stimuli, nor the contrast they provide to the green receptor type account for the values obtained for the first choices, it may be concluded that after very short learning of a neutral signal, flower-naive honeybees evince in their colour choice behaviour innate preferences which put the colour stimuli in a particular rank order. These preferences may be influenced by the pre-training stimulus, but the nature of this influence remains unclear.

Late tests

With increasing experience on the aluminium disk (Fig. 4, squares), bees mostly prefer colour stimuli in the bee green category (around 530 nm) and, secondarily, colours in the uv-blue and blue categories (around 410 nm and 430 nm, respectively). Landings were recorded over four tests preceded by their corresponding learning trials. The null hypothesis of a random distribution of landings on all colour stimuli must be rejected

Fig. 4 Pretraining to achromatic stimuli. "Late tests": percentage of landings on the test stimuli after increased experience on an achromatic pre-training signal (aluminium disk; grey disk; hardboard disk)



ted (1537 landings; log likelihood ratio test: $G = 429.27$; $P < 0.0001$).

Bees that received further rewards on the grey disk (Fig. 4, open circles) also prefer colour stimuli in the bee green category (around 530 nm) and, secondarily, colours in the uv-blue and blue categories (around 410 nm and 430 nm respectively). Landings were recorded over three tests preceded by their corresponding learning trials. The null hypothesis of a random distribution of landings in all colour stimuli must also be rejected here (858 landings; log likelihood ratio test: $G = 248.30$; $P < 0.0001$).

Bees repeatedly rewarded on the hardboard disk (Fig. 4, triangles) clearly prefer colour stimuli in the bee uv-blue, blue and blue-green categories (around 410 nm, 430 nm and 500 nm respectively). In this case, landings were recorded over ten tests preceded by their corresponding learning trials. The distribution of landings significantly differs from a random distribution (1258 landings; log likelihood ratio test: $G = 1153.15$; $P < 0.00001$).

Again, neither perceptual distance to the test background (aluminium disk: $r_s = -0.32$; $n = 12$; NS; grey disk: $r_s = -0.35$, $n = 12$; NS; hardboard disk: $r_s = -0.41$, $n = 12$; NS), nor similarity to the pre-training signal (aluminium disk: $r_s = 0.06$, $n = 12$; NS; grey disk: $r_s = -0.27$, $n = 12$; NS; hardboard disk: $r_s = -0.41$, $n = 12$; NS), nor stimuli intensity (aluminium disk: $r_s = -0.49$, $n = 12$; NS; grey disk: $r_s = -0.57$, $n = 12$; NS; hardboard disk: $r_s = 0.13$, $n = 12$; NS), nor green contrast (aluminium disk: $r_s = 0.30$, $n = 12$; NS; grey disk: $r_s = 0.18$, $n = 12$; NS; hardboard disk: $r_s = 0.24$,

$n = 12$; NS) can account for the colour choice of the naive-bees after increased experience on one of the three achromatic signals.

As before, all three distributions shown in Fig. 4 significantly differ from each other (aluminium vs grey: $G = 88.55$; df: 11; $P < 0.025$; aluminium vs hardboard: $G = 659.71$; df: 11; $P < 0.05$; grey vs hardboard: $G = 342.73$; df: 11; $P < 0.01$). We conclude that colour choice is not independent of the pre-training stimulus and that increased experience on neutral signals may also affect colour preferences of naive honeybee foragers. By comparing Figs. 3 and 4, it may be seen that, even after a prolonged training, the preference for uv-blue and green stimuli persists (around 410 nm and 530 nm, respectively). Apart from the observation that stimuli that are more distant from the pre-trained signals are chosen more frequently, in all cases, increased experience on neutral signals seems to enhance in addition the choice of blue and green stimuli (around 430 nm and 530 nm respectively).

Pre-training to the chromatic stimulus

First test

To compare the effect of pre-training to achromatic stimuli with that of pre-training to chromatic stimuli, an ultraviolet filter (u1: UG1 Schott filter) was selected because u1 (dominant wavelength: 366 nm) has a high spectral purity (Fig. 2) but is rarely chosen by bees after training to a neutral signal (see Fig. 3). The distribution

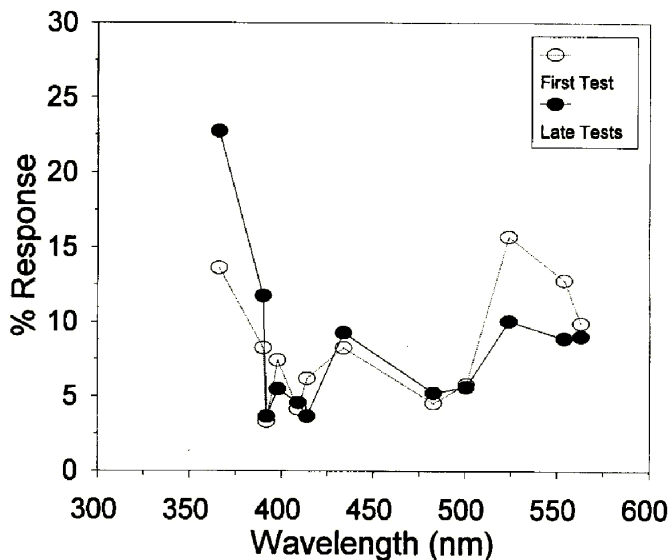


Fig. 5 Pre-training to a chromatic stimulus [ultraviolet filter (u1)]. "First test": percentage of landings on the test stimuli after one reward on the training u1. "Late tests": percentage of landings on the test stimuli after three rewards on the training u1

of landings significantly differs from a random distribution (Fig. 5: empty circles; 242 landings; Log likelihood ratio test: $G = 49.65$; $df = 11$; $P < 0.001$). In their first test, naive bees show a preference for bee-green stimuli (around 530 nm). This preference was even stronger than that for the trained u1 stimulus. The uv-blue and blue stimuli are hardly chosen at all, contrary to the choice frequencies after one reward on neutral stimuli (see Fig. 3). This confirms an observation made above, namely that naive bees tend to choose colours maximally different from the pre-training stimulus, in contrast to experienced bees. However, as opposed to the tests after pre-training with neutral stimuli, here bees show at least some tendency to visit the trained colour.

In this case, again neither the test-stimuli distance to the background ($r_s = 0.15$; $n = 12$; NS), or to the pre-training signal ($r_s = 0.20$; $n = 12$; NS), nor their intensity ($r_s = -0.51$; $n = 12$; NS), nor the specific contrast they provide to the green receptor type ($r_s = -0.51$; $n = 12$; NS), account for the results obtained.

Late tests

Colour preference changes dramatically after repeated training to u1 (Fig. 5: filled circles). Flower naive honeybees that received further rewards on the ultraviolet filter u1 clearly prefer the trained signal, showing that they learn chromatic stimuli and generalize to similar colours more strongly, the better they have learned them (765 landings; Log likelihood ratio test: $G = 235.24$; $P < 0.0001$). In this case, landings were recorded over three tests preceded by their corresponding learning trials. It is clear that the original preference

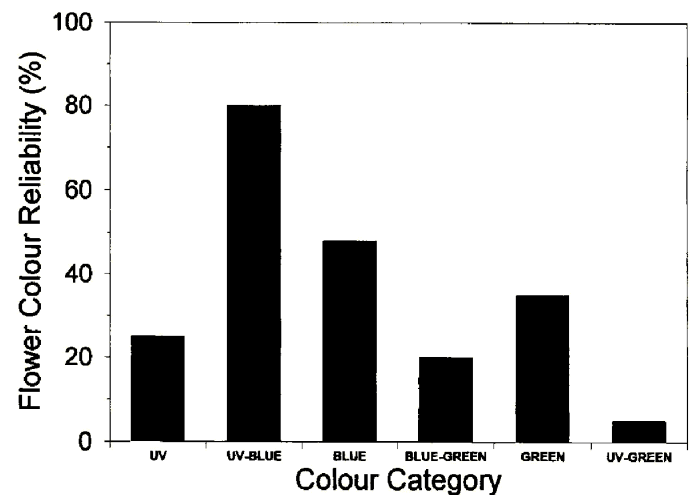


Fig. 6 Flower colour reliability of the colour categories, as measured in 154 flower species

for bee-green stimuli (around 530 nm), which was even stronger in the first test than that for the trained u1, disappears in the third trial, and short wavelength colours are chosen most frequently.

Again, neither the distance of the test stimuli to the background ($r_s = 0.15$; $n = 12$; NS), nor their intensity ($r_s = -0.51$; $n = 12$; NS), nor the specific contrast they provide to the green receptor type ($r_s = -0.22$; $n = 12$; NS) explain the results obtained.

The relationship between colour preferences and floral colour reliability

Figure 6 depicts the results obtained from the study on flower colour reliability (see "Flower colour reliability" in Materials and methods). Different flower colours are very different in terms of their reliability. Uv-blue colours offer a high reward in the highest percentage of cases (80%); blue-green (usually white for humans) and uv-green (human yellow) flowers are the least reliable in terms of this criterion (20% and 5%, respectively). Other colours range in between these two extremes. This trend is not observable when one compares the absolute numbers of high reward colours in each colour category. There are equally many species with high rewards in the blue-green as in the uv-blue category. However, since the total number of species with blue-green flowers is much higher, the probability of hitting a low-reward flower is much higher in the blue-green than in the uv-blue category.

First test

To test whether this pattern might be reflected in the colour preferences of naive bees, manifested after pre-training to the achromatic stimuli, we assigned the

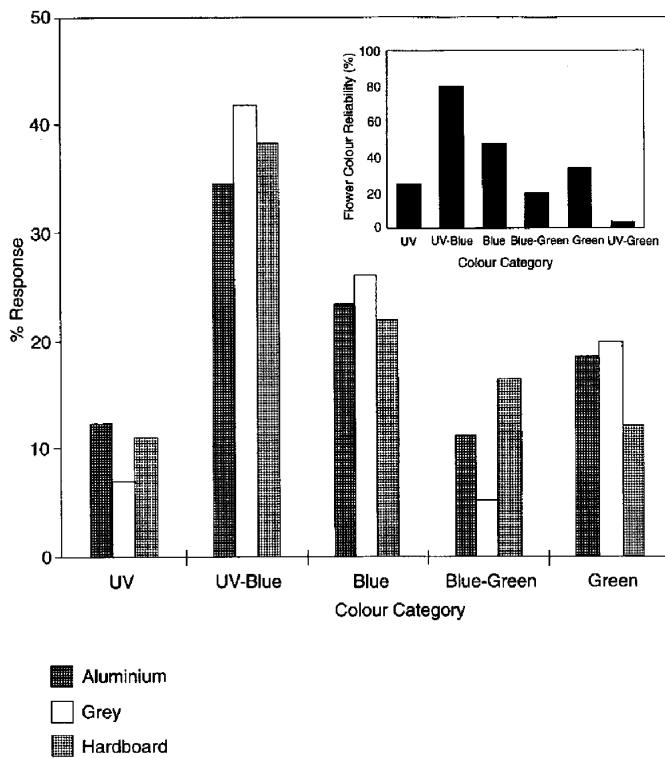


Fig. 7 "First test": percentage of responses on the test stimuli after one reward on an achromatic signal (aluminium disk; grey disk; hardboard disk), grouped following the bee colour categories (for terminology see Chittka et al. 1994). Inset: flower colour reliability (from Fig. 6)

flower colours and their reliabilities to the six colour categories used to group the test stimuli (see Fig. 2). Since the UV-green category contains no colour loci, we can only compare the flower colour reliability of the remaining five colour categories with the respective pooled choice frequencies of the first test, for test stimuli that lie within these same categories (Fig. 7). Since these choice frequencies differ depending on the pre-training stimulus (see Fig. 3), the correlation with the colour reliability was determined separately for the three sets of data. For the sake of comparison, the inset of Fig. 7 depicts the flower colour reliability from Fig. 6.

From the three bar diagrams shown in Fig. 7, those corresponding to the pre-training with the aluminium and the grey disks significantly correlate with that representing the flower colour reliability (inset) (aluminium disk: $r_s = 1$; $n = 5$; $P < 0.02$; grey disk: $r_s = 1$; $n = 5$; $P < 0.02$), whereas that corresponding to the pre-training with the hardboard disk does not show a significant correlation with the flower colour reliability (hardboard disk: $r_s = 0.70$; $n = 5$; NS). Note, however, that even in the latter case, both bar diagrams follow a similar pattern of variation, with the uv-blue category being more frequently chosen, followed by the blue one. Thus, the coincidence between flower colour reliability and the curve representing the wavelength

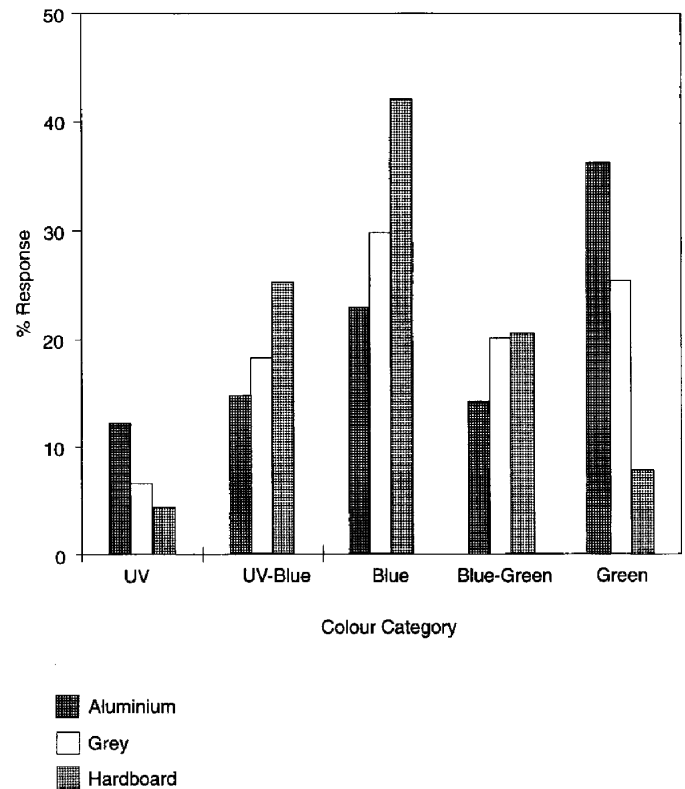


Fig. 8 "Late test": percentage of responses on the test stimuli after increased experience on an achromatic signal (aluminium disk; grey disk; hardboard disk), grouped following the bee colour categories

dependency of the first choices of naive bees suggests that these choices represent an innate tendency to search for flowers according to their reliability to signal nectar reward.

Late tests

The same procedure was followed for results of the late tests. Figure 8 depicts the values obtained when representing our data according to the colour categories. None of the bar diagrams correlates significantly with that representing the flower colour reliability (Fig. 6) (aluminium disk: $r_s = 0.50$, $n = 5$; NS; grey disk: $r_s = 0.20$, $n = 5$; NS; hardboard disk: $r_s = 0.60$; $n = 5$; NS). Increased experience on neutral signals enhances the choice of blue and green colours, thus changing the original preference pattern (Fig. 7).

The relationship between colour preferences and the learning speed of colours in conditioning experiments

Since a rank order of colour choice was also obtained by Menzel (1967) in learning experiments with experienced honeybee foragers, we next raised the question of whether the colour stimuli preferred by naive foragers

have not only a natural correlate in floral coloration and its associated nectar reward, but are related in addition to the wavelengths that experienced foragers learn with different speeds in conditioning experiments (Menzel 1967).

First test

In Fig. 3 (inset) the wavelength dependence of the learning process as obtained by Menzel (1967: Figure 9) is given by plotting the choice percentage in a dual choice situation after a single learning trial. In this case, bees were rewarded once on a particular colour and tested thereafter with two unrewarded colours, one of which was the training one. Whereas this wavelength dependence depicts the learning performance of colour stimuli after one learning trial, the three curves of Fig. 3 represent the defined wavelength dependence as measured by the first choices without particular colour experience. All three curves significantly correlate with that obtained in learning experiments (aluminium disk: $r_s = -0.77$; $n = 12$; $P < 0.005$; grey disk: $r_s = -0.73$; $n = 12$; $P < 0.01$; hardboard disk: $r_s = 0.66$; $n = 12$; $P < 0.05$). The association between Menzel's results (Fig. 3, inset) and flower colour reliability (Fig. 6) was tested following the same procedure. Again, the two variables are significantly correlated ($r_s = 0.94$; $n = 6$; $P < 0.05$).

Thus, uv-blue and green colours (around 400 nm and 530 nm, respectively) are more frequently chosen by flower-naive honeybees, are also learned faster by experienced honeybees, and correspond to flowers with the highest nectar reliability.

Late tests

We compared our data (Fig. 4) with those obtained in colour learning experiments (Menzel 1967) (Fig. 3: inset). In this case, no significant correlation was found (aluminium disk: $r_s = -0.12$, $n = 12$; NS; grey disk: $r_s = -0.08$, $n = 12$; NS; hardboard disk: $r_s = 0.38$; $n = 12$; NS). Increased experience on the three achromatic signals thus seems to erase the original preference pattern that coincides with Menzel's results.

Discussion

The present work examines the colour choice of bees that have had no experience with natural flowers prior to testing them with a variety of coloured stimuli presented simultaneously, and demonstrates the existence of colour preferences. Flower-naive foragers, whose previous colour experience was kept to a minimum, preferred stimuli of the colour categories bee uv-blue

and green (dominant wavelengths around 410 nm and 530 nm, respectively). These colour preferences resemble those known from learning studies with colour signals (Menzel 1967; see inset in Fig. 3) and have a natural correlate in flower coloration and its associated nectar reward (Fig. 7).

Another interpretation is, however, possible, namely that spontaneous preferences are restricted to colours in the green category and that preferences for uv-blue colours reflect in fact a learning process. In tests following "neutral" pre-training, the bees preferred uv-blue in all cases (Fig. 3), even after a prolonged training on these stimuli (Fig. 4). Since, the aluminium and grey stimuli are shifted to the uv-blue category in relation to the test background (see Fig. 2), it may be argued that the uv-blue preference, after "neutral" pre-training, is due to the pre-training itself, whether one reward or several are given prior to the test. Uv-blue colours are indeed learned very fast (see inset in Fig. 3), even after a single reward (Menzel 1967). If the bees' preference for uv-blue colours were spontaneous, then one would expect it to manifest even in the chromatic uv pre-training, particularly in the first test, where the green preferences are visible (Fig. 5: empty circles). However, no uv-blue preference was detected in this case. This would indicate that uv-blue is learned very fast, even when it is very unsaturated, and that the preference for green is the only innate one. The interpretation is attractive due to its evolutionary and ecological implications: unexperienced bees would head toward foliage and would, once they have arrived there, be attracted by the scent of the flowers (see in Materials and methods that no bee alighted on unscented colour disks; see also below). However, this would not explain why bees also preferred uv-blue colours after pre-training with the hardboard disk, whether one or several rewards are given. This achromatic pre-training signal neither reflected particularly in the uv-blue region, nor was shifted to the uv-blue corner of the colour space. Also in the case of aluminium- and grey-disk pre-training, if uv-blue were learned, one would expect to find a significant rank correlation between the stimuli choice frequencies and the colour distance to the pre-training signals aluminium and grey. We did not find such a correlation (see Results). Moreover, if the preference for uv-blue were the result of a learning process, bees would have chosen preferentially in tests the u-b2 stimulus (see Table 1: dominant wavelength 392 nm) after pre-training to aluminium (dominant wavelength 393 nm) and the u-b3 stimulus (dominant wavelength 398 nm) after pre-training to grey (dominant wavelength 402 nm), in the first as well in the late tests. This never occurred. Thus, our experiments actually reveal colour preferences on the part of the bees for uv-blue and green stimuli. These preferences are interpreted as innate predispositions to spontaneously choose, and afterwards learn faster, certain colours as food signals because the rank order of preference can-

not be explained through the following perceptual properties of the colour stimuli tested 1) their perceptual distance to the background (spectral purity) or 2) to the pre-training signal, 3) their intensity or 4) their specific contrast to the green receptor. Hence bees have indeed an innate preference for certain hues over others.

1) Colour distance to the background, for instance, was not used as spectral parameter determining the bees' choices. The theory of colour vision in honeybees (see Backhaus 1993 for review) assumes that the three kind of photoreceptors that bees possess, ultraviolet, blue and green, are adapted so that they render half their maximum response when exposed to the light reflected from a given background (Backhaus and Menzel 1987). In our experiment, the test stimuli were presented against the hardboard plate which thus constitutes the adaptation light and comes to lie in the centre of the colour space. As explained, bees may have chosen the test stimuli that were more detectable against the hardboard background, i.e. the stimuli with a greater perceptual distance to the background. This was not the case and no significant correlation was found. 2) This leads to the second parameter analyzed, the perceptual distance to the pre-training stimuli. Though the pre-training stimuli aluminium, grey and hardboard were achromatic, if bees memorized them during the pre-training procedure and then tried to match the new perceived test stimuli with the memorized ones, aluminium and grey lie now on loci that slightly differ from the central point of the colour space, due to the adaptation process described above (see Fig. 2). Only in the case of the hardboard disk, which was at the same time pre-training stimulus and test adaptation light, there is a perfect coincidence. Thus, bees may have chosen the test stimuli with a greater perceptual similarity to the pre-training signals. This was not the case, as demonstrated (see Results). Only in the case of UV pre-training, the effect of colour perceptual similarity to the pre-training stimulus manifested after three rewards (Fig. 5: filled circles). 3) Intensity differences have already been shown to be irrelevant for experienced foragers in colour discrimination tasks (Daumer 1956; Menzel 1967; Backhaus et al. 1987; Backhaus 1991, 1992; Brandt et al. 1993; Giurfa 1991; Chittka et al. 1992); the present study shows that this is also true in the case of naive bees. 4) The green-sensitive photoreceptors, and therefore green-contrast, have been shown to play a major role in motion-dependent visual guidance (rev. Lehrer 1987, 1993). The present results show that green contrast plays no role in a task that does not require the use of motion cues. Indeed the exclusive use of the green receptor would result in colour blindness. While a colour blind system could discriminate among different intensity values, it is not expected to differentiate among colours.

It is noteworthy, however, that totally naive honeybee foragers were never attracted by colour stimuli,

unless they have previously been rewarded on a scented stimulus. The fact that the bees were ready, after only one reward on a scented stimulus (whether chromatic or achromatic), to make choices among a variety of similarly scented coloured stimuli, can only be due to the scent. This has two important implications: 1) in behavioural terms this means that odour is the primary factor that attracts naive bees and thus, that a bee's first encounter with a flower cannot be facilitated by colour alone. Colour is certainly only one component in the full, multisensory search image applied by bees on their first foraging flight. 2) in experimental terms this means that we had to reward the bees at least once in order to make them choose colour stimuli. The subsequent choices were clearly influenced by the properties of the once rewarded stimulus. The nature of this influence, at least in the achromatic pre-training stimuli, remains unclear. The rank order of choices was not related to their similarity to the pre-training stimulus, as in the case of experienced bees (Daumer 1956; Menzel 1967; von Helversen 1972; Backhaus et al. 1987), even when trained to achromatic stimuli (Giurfa 1991). Indeed our bees tended to select stimuli that were maximally different from the pre-training stimuli. This discrepancy in the colour choice behaviour between naive and experienced bees suggests that naive bees follow different rules than do experienced bees.

Interestingly, our results allow us to solve a traditional dispute about the spontaneous colour preferences of honeybee foragers. For example, Ludwig (cited in von Frisch 1967), Menzel et al. (1973) and Gould (1984) suggest that foragers prefer human blue colours (here bee uv-blue and blue colours). In contrast, Oettingen-Spielberg (1949) and Real (1981) affirm that they prefer human yellow stimuli (here bee green colours). Finally, Butler (1951) maintains that both of these colours are chosen equally by honeybees. Although in all cases experienced foragers were used, thus making the use of the term "spontaneous preferences" inadequate (see Introduction), it is interesting to find that uv-blue and green predominate over other possible hues. According to our results, both uv-blue and green are preferred by naive honeybee foragers and biases to one or the other may occur according to the pre-training procedure and the level of experience of the individuals tested.

Studies on naive bumblebees revealed no preference for particular colours, but a tendency to prefer more spectrally pure colours over those that are closer to the uncoloured point (Lunau 1990, 1991, 1992). Whilst we find no direct support for this hypothesis in honeybees, there is some evidence that the parameter of spectral purity plays a role in learning a colour stimulus. Achromatic stimuli are not learned in such a way that perceptually similar stimuli are chosen more frequently, whereas ultraviolet stimulus training leads to an increase in the frequency with which it and perceptually similar stimuli are chosen. Concurrently, the initial

preference for the green stimuli decreases. This observation is supported by Daumer (1956), who found it more difficult to train bees to mixtures of light with a high component of white light.

This brings us back to the relationship between colour choices of naive bees and the learning speed for different colours. Interestingly, the results that predicted the innate preferences of bees most accurately were obtained with experienced bees (Menzel 1967). This study showed that bees learn faster those colours that are also preferred by naive bees in our study: uv-blue, blue and green. Although it has always been conjectured that these results reflected an innate predisposition for learning these particular colours (Menzel 1967, 1985), there remained the possibility that the bees had been guided by their previous experience in the field that was unknown to the experimenter. Already in Menzel's study (1967), an important argument was that the acquisition functions for spectral colours did not change over the course of the year, i.e. under conditions of different colour learning backgrounds of the bees tested. Moreover, our method clearly excludes the possibility that bees were influenced by previous foraging experience. We find that indeed the evaluation of colour learning speed even in experienced bees renders a good measure of their innate preparedness for learning particular colour signals.

For the first time, we offer an ecological interpretation for bee colour preferences. The colour choices of bees, as well as the learning speeds for different colours, correlate well with the nectar production of flowers of different colours. This means that the innate search image of bees for flowers does not only allow the bees to differentiate a potential "floral" object from a "non floral" object, but also to anticipate the probability of finding a high nectar reward at flowers of particular colours.

No flower colour, however, is an absolute guarantee for high reward. Even the uv-blue flower category (the one with the highest reliability; Fig. 6) contains low-reward flowers, and there are numerous species with blue-green flowers that have high rewards. In addition, we investigated only one particular set of flowers, while the informational content of flower colours may well differ among habitats. With this in mind, the innate preferences should only facilitate the first encounters of naive bees with a flower. Later, the learned relationship between particular signals and the rewards found in association with these should override the innate preferences. There is indeed some evidence that this is the case. Menzel (1967) found that after only a few learning trials, all colours were chosen with equal precision, i.e. the initial preference pattern had vanished.

Other studies, however, contend that bees prefer blue colours with low rewards even after extensive training on other colours with higher rewards (Heinrich et al. 1977; Banschbach 1994). Our study contains a similar

result: even after several rewards, bees almost never chose the stimuli that are perceptually close to the achromatic stimuli on which they were rewarded. This behaviour is clearly suboptimal in our experimental setup. Even though other results indicate that choice rates at artificial flowers are more or less matched to the rewards and independent of a particular colour signal (Greggers and Menzel 1993), these results hint that the equilibrium between innate preferences and learning performances, which has to be adjusted evolutionarily in each species according to its particular needs, may not always be optimal under all conditions.

Finally, it remains to ask how the observed preferences are neurally encoded. Clearly, the mechanism that bees use for colour discrimination, colour opponent coding (Kien and Menzel 1977; Backhaus 1991, 1993; Chittka et al. 1992), cannot account for their preparedness for particular colours. In other words, we understand what allows bees to distinguish among colours, but we do not know why blue and green are evaluated more strongly as food signals than are other colours. Still, our results, when viewed together with those obtained by Menzel (1967), render evidence that different colours have indeed different meanings for bees. This in turn means that higher perceptual colour phenomena, such as unique hues, are likely to exist in bees. Obviously, such capacities must somehow be neurally encoded. However, where in the brain and with which mechanisms this is accomplished is as yet unknown.

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