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Colour choices of naive bumble bees and their implications for colour perception

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Abstract The innate preferences of inexperienced bumble bees, *Bombus terrestris*, for floral colour stimuli were studied using artificial flowers. The artificial flowers provided a colour pattern and consisted of a star-shaped corolla and of central colour patches similar to the “nectar guide” of natural flowers. The innate choice behaviour was assessed in terms of the number of approach flights from some distance towards the artificial flowers and the percentage of approach flights terminating in antennal contact with the floral guide. The colours of the floral guide, the corolla and the background were varied. It was shown that the innate flower colour preference in bumble bees has two components. 1. The frequency of approaches from a distance is correlated with the colour difference between the corolla and the background against which it is presented. If the corolla colour was constant but its background colour varied, the relative attractiveness of the corolla increased with its colour difference to the background. The colour difference assessment underlying this behaviour on a perceptual basis can be attained by means of colour opponent coding, a system well-established in Hymenoptera. 2. The frequency of antennal contacts with the floral guides relative to that of approach flights cannot be accounted for by colour opponent coding alone. Whether the approach flights are interrupted, or whether they end in an antennal

contact with the “nectar guide” is strongly dependent on the direction (sign) of the colour difference, not only its magnitude. The choice behaviour requires a unique perceptual dimension, possibly that of colour saturation or that of hue perception comparable to components of colour perception in humans.

Key words *Bombus terrestris* · Innate colour preferences · Colour difference · Colour saturation · Colour vision · Colour perception

Introduction

The capacities of Hymenopterous insects in associating arbitrary colours with rewards are intriguing (von Frisch 1915; Menzel and Backhaus 1991; Chittka et al. 1992). However, prior to individual experience with natural flowers, freshly emerged flower-visitors need to distinguish potential food sources – i.e. floral signals including a wide range of different colours – from non-floral objects, such as green leaves, stones, sand, autumn leaves, etc. (Giurfa et al. 1995). Once a floral signal has been detected, probing time can be minimized if the flower-visitor has an innate program that allows it to locate the site of the reward (Osche 1983; Lunau 1990, 1991a,b; Laverty 1994).

A conceivable concept to distinguish flowers of several colours from the background and to orientate at floral colour patterns might be to use an evaluation of colour saturation (Lunau 1990). The perception of colour saturation implies that the subject can extract the achromatic component of a colour (Evans 1974; Wyzecki and Stiles 1982; Endler 1990). Such a perceptual parameter would be useful for the above task, since all natural backgrounds, such as green leaves and stones are of low colour purity for a bee, whereas the vast majority of flower colours are not (Daumer 1958; Kevan 1978; Endler 1993; Chittka et al. 1994). Indeed,

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Exner and Exner¹ (1910) observed "... that we can discern manifold designs in flower petals which generate a relatively high colour saturation. Thus we must ... conclude that the more saturated colours are more conspicuous than unsaturated ones for insects ... as it is the case for us. Only the most brilliantly coloured jewels surpass certain flower colours in colour saturation." (translated from German by the authors—original wording see footnote 1).

At the time little was known about insect colour vision being essentially unlike that of humans. We now know that the visual ranges of insects are very different both in different species and from our own (Menzel and Backhaus 1991; Peitsch et al. 1992) and that colour perception may differ between humans and insects (Wyszecki and Stiles 1982; Backhaus 1991; Chittka et al. 1992, 1994). These observations caution against assuming that the dimensions present in human colour perception exist in any animal species; their existence needs to be explicitly shown by means of critical experiments. So far, the perception of colour saturation has not been explicitly demonstrated in an insect.

Do bumble bees detect floral (as opposed to non-floral) signals by evaluating their colour saturation? Using artificial flowers, Lunau (1990, 1993) demonstrated that the innate colour preference in naive bumble bees is correlated with the distance between the loci of the colour stimuli and the locus of the uncoloured point, the centre of the colour space which represented a stimulus with constant reflection. The frequency of approach flights towards artificial flowers was correlated with the distance between the locus of the corolla colour and the uncoloured point. The percentage of approaches terminating in antennal contact with the floral guide was correlated with the distance between the locus of the floral guide colour and the uncoloured point *minus* the distance between locus of the corolla colour and the uncoloured point, i.e. higher absolute difference values caused higher frequencies of approaches terminated by antennal contact with the floral guide. In contrast to the spectral purity, the stimulus intensity and dominant wavelength had no detectable influence on the choice behaviour, whereas the contrast between corolla colour and floral guide colour had some influence. These results might be explained by the assumption that bees possess the perception of colour saturation, but are they a proof of its existence or are there alternative explanations?

In a preliminary study, Chittka and Lunau (1992) demonstrated that the choice behaviour can also be

explained using a more parsimonious explanation of a perceptual colour space which plots the 3 photoreceptor excitations and which is a general representation of colour opponency, a colour coding system well established in several genera of Hymenoptera (Chittka 1992; Chittka et al. 1992). Chittka and Lunau (1992) analyzed the data from Lunau's multiple choice test (1990) to test whether the bumble bees might have simply evaluated colour differences between the corollae and the background, and between floral guides and corollae. Indeed, these authors found this to be a likely explanation. The question whether bees possess the perception of colour saturation remained open.

In the present study we re-analyze Lunau's (1990) behavioural data in order to summarize the evidence with respect to this question. Moreover, new critical experiments were designed to critically separate these parameters, spectral purity and colour difference. These experiments are critical to test whether the colour choice behaviour of naive bumble bees can be explained by colour opponent coding alone, or whether any additional capacity of colour perception is a necessary addition to contemporary concepts of bee colour perception. In one of these experiments, the bumble bees are tested at pairs of colour combinations of background, corolla and floral guide which were reciprocal. In these pairs of artificial flowers the colour difference (as quantified by a colour opponent system) is held constant, whereas the spectral purity pattern is reversed. Thus the direction of the colour difference (sign) is different, but not its magnitude. Since bumble bees responded differently to the two directions of each pair of colour combination, we assume that they possess a unique perceptual dimension that enables them to evaluate the direction of colour difference.

Materials and methods

Rearing of bumble bees

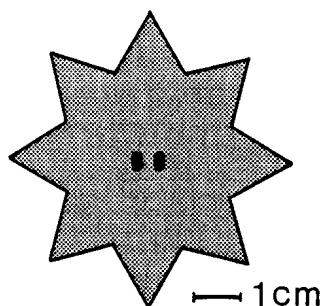
The worker bumble bees were housed in a flight cage. They had been reared in the laboratory from captured queens. The bumble bees were inexperienced with natural flowers, and were never rewarded at artificial flowers, neither before nor during the behavioural tests. Pollen was fed directly into the nests and diluted honey was permanently offered in transparent feeders close to the nest-entrance. The workers of one colony were tested simultaneously. For further details see Lunau (1990).

Experimental setup

The star-shaped artificial flowers (ϕ 56 mm) were presented on vertical experimental tables. These tables (50 cm * 50 cm) provided the background colour. The artificial flowers consisted of a corolla and of a floral guide. The floral guide consisting of two ovally shaped dots (each 2 mm * 4 mm) was located in the center part of the corolla (Fig. 1). Coloured cardboards (spectral reflection, see Fig. 2) were used to construct the artificial flowers. At each experimental table,

¹ Exner and Exner (1910): "... dass wir allerlei Einrichtungen an den Blumenblättern kennenlernen werden, welche eine relativ grosse Sättigung ihrer Farben bewirken. Folgerichtig müssen wir aus den vorstehenden Betrachtungen entnehmen, dass fuer das Insekt diese gesättigten Blütenfarben auffallender sind als ungesättigte ... wie das bei uns der Fall ist ... Nur die farbenprächtigsten Edelsteine uebertreffen noch gewisse Blütenfarben an Sättigung."

Fig. 1 Outline of the artificial flowers with star-shaped corolla and floral guide in the centre of the corolla. *White*: background; *grey*: corolla; *black*: floral guide



two artificial flowers were offered, 20 cm apart. From 3 to 6 experimental tables were offered at a time, sometimes with identical colour combinations (for example: separate testing in Exp. 3). The colours of the floral guide, of the corolla and of the background were varied. Daylight was simulated by the illumination light (Exp. 1: OSRAM L25/40W running on direct current; Exp. 2 and 3: OSRAM BIOLUX L58/W72 running on 30 kHz, alternating current). The experimental tables as well as the artificial flowers on each experimental table were alternated in position every 15 min in order to avoid the effects of possible positional preferences. In this way, in far most cases the positions had been changed between subsequent series of visits of individual bumble bees.

Analysis of colour stimuli

The spectral reflection of the artificial flower colours was recorded from 300–700 nm with a diode array spectrometer using a single beam ZEISS MCS 230 photometer unit, a Zeiss CLX 111 Xenon light source, and a MS 20 ceramic as white standard (reference of 100% reflection) (Fig. 2). The loci of the artificial flower colours of Exp. 1 in the colour triangle were shown by Lunau (1990).

The spectral reflections of the artificial flower colours [$R_s(\lambda)$] are weighted by the spectral emission of the flight cage illumination [$\Phi(\lambda)$], and by the spectral sensitivity of the three types of photoreceptors [$r_{s,M,L}(\lambda)$] (in the short, middle and long wavelength regions) of *Bombus terrestris* recorded by electrophysiological methods (Peitsch et al. 1992). The effective quantum catch for a given stimulus in the types of photoreceptors was calculated according to

$$P = R \int_{300}^{700} r_s [r_M, r_L] (\lambda) * R_s (\lambda) * \Phi (\lambda) d\lambda. \quad (1)$$

The range sensitivity R depends on the adaptation state. The photoreceptors are assumed to be adapted to the background colour. The adaptation process is assumed to adjust each receptor's sensitivity so that it renders half its maximal response when stimulated by the light reflected from the adaptation background (Laughlin 1981). The calculation of the quantity R in equation (1) follows:

$$R = 1 / \int_{300}^{700} r_s [r_M, r_L] (\lambda) * R_B (\lambda) * \Phi (\lambda) d\lambda. \quad (2)$$

R_B is the spectral reflection function of the background.

The background tables on which the artificial flowers were presented measured only 50 cm * 50 cm and we did not quantify for how long time the bees hovered in front of this background colour. Thus it remains unclear whether the photoreceptors retained the adaptation state resulting from the light reflected from the overall background in the flight room, or whether they were already, at least partially, adapted to the background tables. These considerations are critical for the second and third of the present experiments, because the colour of the background tables is varied (see below).

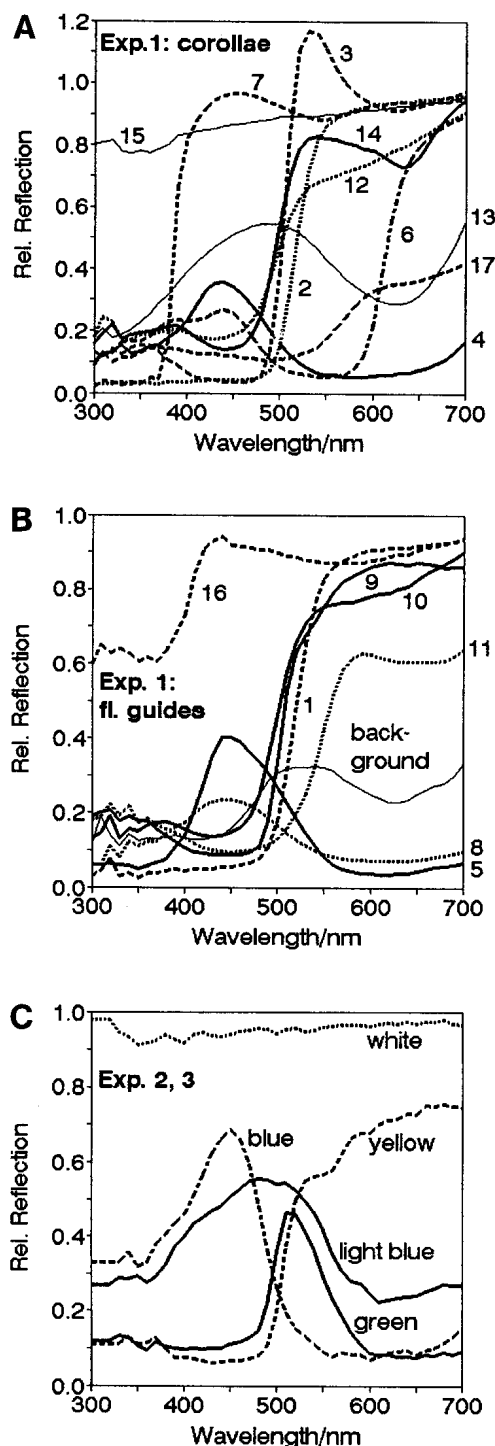
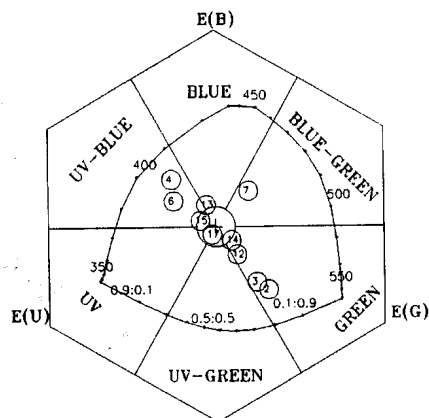


Fig. 2A–C Spectral reflection of artificial flower colours. **A**, **B** background colour, corolla colours and floral guide colours of Experiment 1. **C**: all colours of Experiment 2 and Experiment 3

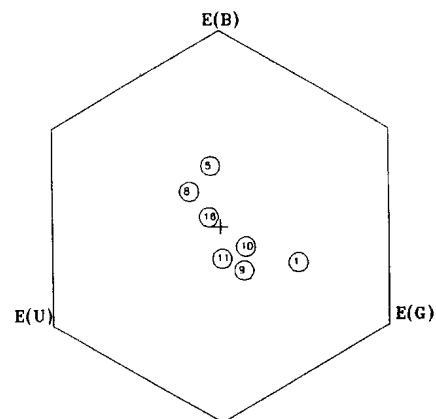
Since the photoreceptors represent the interfaces between stimuli and colour perception, all perceptual phenomena, including colour saturation, depend on the relative sensitivities of the photoreceptors. It is well known that the adaptation states are influenced by the spectral composition of ambient light (Laughlin 1981; Wysocki and Stiles 1982). In our experiments the bumble bees view the coloured artificial flowers with the ventral and frontal eye regions; these are presumably adapted to the spectral composition of the ambient light

Fig. 3 Loci of the artificial flower colours (circles) in the colour hexagon. The figure (upper left) shows the operational colour category boundaries according to which our stimuli are named. The central circle encloses the uncoloured category. The continuous line represents the spectrum locus at background intensity. Points are calculated in 10 nm steps and labelled every 50 nm. The short and long wavelength ends of the spectrum locus are connected by the UV-green mixture line in 9 mixture ratios. The uncoloured point is marked by a cross in all 4 diagrams. For Experiment 1, colour loci are calculated assuming that the photoreceptors are adapted to the green background tables. They are numbered according to their ranks in spectral purity. For Experiments 2 and 3, colour loci were calculated for the same adaptation model as for Experiment 1 (Figs. 6, 7) and for the assumption that the receptors were adapted to the overall colour of the flight cage wall (shown in this figure)

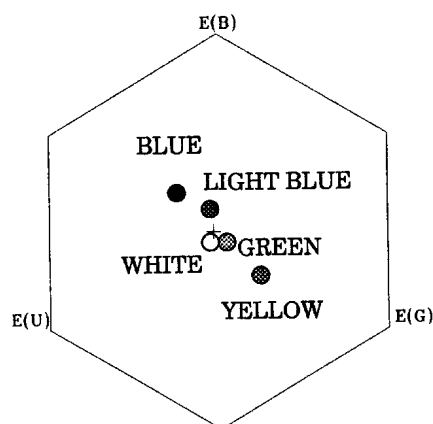
EXP. 1: COROLLA COLOURS



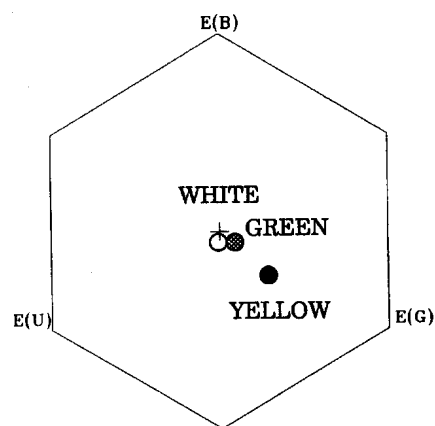
EXP. 1: GUIDE COLOURS



EXP. 2: COROLLAE & BACKGND.



EXP. 3: ALL COLOURS



reflected from the walls and floor of the room. Our model assumption for adaptation—that receptors display half their maximal response when adapted to a background (Laughlin 1981) has recently been confirmed by means of behavioural experiments for all but strongly chromatic backgrounds (Dittrich 1995). With this assumption, the adaptation colour shifts into the centre of the colour diagram, and saturation is referred to as the distance to that locus. It was not quantified how long the bumble bees hovered in front of the experimental tables on which the dummies were presented; however, it is conceivable that this table background might have influenced the relative sensitivities of the photoreceptors. To be sure that our interpretation that bumble bees responded not only to colour difference was not an artifact of an adaptation assumption that ignored the table background, we recalculated the colour loci for the extreme assumption that receptors were adapted exclusively to the presentation tables. Following equation (2), the adaptation colour shifts into the centre of the colour diagram. The adaptation assumptions are henceforth called “room adaptation” and “table adaptation”.

The calculation of the physiological voltage signals (excitations E) from quantum catch values P is described by

$$E = P / (P + 1). \quad (3)$$

For more details see Chittka et al. (1992). The conversion of excitation values into colour hexagon coordinates follows Chittka (1992). The hexagon loci of all colours used in Experiment 1 are given in Fig. 3. In this experiment, both the colour of the cage floor and walls as well as the colour of the background tables were green (Fig. 2) so

that the position of the colour loci was identical for both adaptation hypotheses. Figure 3 shows the colour loci for “table adaptation”. For Experiments 2 and 3, Fig. 3 depicts the colour loci for “room adaptation”, whereas Figs. 6 and 7 show the loci for “table adaptation”. Artificial flower colour were given operational bee colour names depending on where their loci were placed in the colour hexagon, and given the prefix “bee”. Colour loci with a distance < 0.1 from the centre of the colour hexagon are termed “bee-uncoloured” (cf. Chittka et al. 1994). The colours as perceived by humans are indicated without prefix. Colour distances in the colour hexagon will be operationally termed “colour difference” and represent the geometric distance between two colour loci. To denote the distance of a given colour locus to the uncoloured point, we use the term “spectral purity” in an operational sense, without making any assumptions about whether bees perceive this particular direction (sign) of colour difference as unique.

Behavioural experiments

The bumble bees landed only very infrequently on the artificial flowers, probably because olfactory releasers of the landing reaction were not present (Lunau 1991b). Thus, we evaluated two different behavioural reactions towards the artificial flowers: 1. The *approach flight* towards an artificial flower usually started from a distance of approx. 1 m. The bumble bees may interrupt the approach flight before they make contact with the artificial flower, or continue the

Table 1 Experimental set-up. For each experiment, the background colour, corolla colour and floral guide colour of the artificial flowers are shown; the numbers and colour names refer to the figures in which the spectral reflection and hexagon loci are shown

Experiment number	Experiment table colour	Corolla colour	Flora guide colour	n dummies
1A	background	2,3,4,6,7,12,13,14,15,17	1	10
1B	background	2,3,4,6,7,12,13,14,15,17	5	10
1C	background	2,3,4,6,7,12,13,14,15,17	8	10
1D	background	2,3,4,6,7,12,13,14,15,17	9	10
1E	background	2,3,4,6,7,12,13,14,15,17	10	10
1F	background	2,3,4,6,7,12,13,14,15,17	11	10
1G	background	2,3,4,6,7,12,13,14,15,17	16	10
2	white green light blue	blue, yellow blue, yellow blue, yellow	— — —	6
3A	white	green	yellow	2
test 1	white	yellow	green	
3A	green	white	yellow	2
test 2	green	yellow	white	
3A	yellow	white	green	2
test 3	yellow	green	white	
3B	white	green	yellow	6
	white	yellow	green	
	green	white	yellow	
	green	yellow	white	
	yellow	white	green	
	yellow	green	white	

approach up to contact with the artificial flower. 2. The *antennal contact* with the artificial flower was exhibited at the floral guide or at the edge of the corolla. In Experiment 3, the bumble bees interrupted many approach flights towards artificial flowers which were offered against green and yellow backgrounds. We recorded therefore whether the bumble bees interrupted the approach flights, or whether they finally made antennal contact with the artificial flower. The colours of the floral guide, of the corolla and of the background were varied in order to study their influence upon the innate choice behaviour. The innate choice behaviour was assessed in terms of the frequency of approach flights towards the artificial flowers and of the proportion of approaches followed by antennal touches with the floral guide out of the total number of approaches.

Three experiments were performed. The spectral reflection of the artificial flower colours are shown in Fig. 2. The experimental set-ups are shown in Table 1.

Experiment 1

This experiment was performed by Lunau (1990) (see above). Ten corolla colours were simultaneously tested in 7 tests (A–G). In each test, this set of 10 corolla colours was combined with a different floral guide colour. Thus 70 combinations of floral guide and corolla colours were tested in total. In order to facilitate identification the colours were given numbers used in Figs. 2, 4, and in Table 1. The tested bee-blue, bee-green, bee-UV-blue, bee-blue-green and bee-uncoloured colours differed greatly in their spectral purity. The artificial flowers were offered against a green background (Figs. 1, 2, 3).

Experiment 2

One set of two artificial flowers was offered against different backgrounds such that the colour difference between flowers and background varied. Details: The bumble bees were offered three different background tables simultaneously. The background colours tested

were white (bee-uncoloured), light blue (bee-uncoloured), and green (bee-uncoloured); Fig. 3 (“room adaptation”). For “table adaptation”, all background colours are also bee-uncoloured (Fig. 6). On each of these tables, the same pair of two different colours were offered, a blue (bee-UV-blue) and a yellow (bee-green) artificial flower. In this experiment, only the approach flights and not the antennal contacts were evaluated. The arrangement of the artificial flowers and the loci of the tested colours in the colour hexagon are shown together with the results of the experiment (Fig. 6).

The rationale of this experiment is to critically separate the two possible interpretations for bumble bee colour choices outlined in the introduction. For the “room adaptation” hypothesis, we predicted that, if choice frequencies were entirely dependent on spectral purity (distance from the uncoloured point), the ratio of approaches to the two identical stimuli should be independent of colour difference to the local background. If, on the other hand, this ratio changes when the local background is changed, it is more likely that bee assessed colour difference of a stimulus against its background when approaching from a distance. Clearly, for the “table adaptation” hypothesis (that photoreceptors are entirely adapted to the background tables) we cannot distinguish between these two hypotheses, since under these conditions the local background becomes identical with the uncoloured point (the centre of colour space). Thus, colour difference to the background and spectral purity bear identical values. However, this extreme assumption is unlikely to hold in this experiment in which only approach flights from a large distance towards the coloured targets are evaluated. The “table adaptation” assumption is more relevant in the subsequent experiment where bee behaviour at close range to the targets is evaluated. Nevertheless, to allow the reader an assessment of the rearrangement of colour loci under this assumption, these are shown in Fig. 6.

Experiment 3A

Reciprocal colour combinations of background and corolla as well as of corolla and floral guide were offered. In these pairs of artificial

flowers the colour difference is held constant and the spectral purity pattern is reversed. Details: Six colour combinations in which the background, corolla and floral guide colour were varied were tested using 3 colours white, green and yellow. The white colour showed a constantly high reflection of 90% throughout the bumble bee-visible spectrum. The green colour had one reflection peak of 50% at 520 nm, the yellow colour showed strong reflection > 60% at wavelengths > 510 nm. In three separate tests (test 1–3), each of these colours was offered as the background colour together with two artificial flowers which were constructed using the remaining two colours. The two artificial flowers were coloured reciprocally with respect to corolla and floral guide. The three colours were specifically selected so that they differed in their spectral purity. The locus of the white colour is close to the uncoloured point. The green colour has a somewhat higher distance from the very centre of colour space. The white and green colours are bee-uncoloured. The yellow colour is bee-green and has a higher spectral purity than the other two colours (based on the “room adaptation” assumption; Fig. 3). The arrangement of the artificial flowers and the loci of these colours in the colour hexagon (“table adaptation”) are shown together with the results of the experiment (Fig. 7). The various colour combinations thus included reciprocal colouration with respect to corolla and background as well as with respect to floral guide and corolla. By means of reciprocal colouration, the magnitude of the colour difference is left constant, while the direction (sign) is reversed.

Experiment 3B

In a separate test all 6 colour combinations were offered simultaneously. In this test only the approach flights were evaluated, because the number of antennal contacts with the guide was too small for statistical analysis.

Results

Experiment 1

Figure 4 shows the frequency of approach flights towards the artificial flowers offered simultaneously and the portion of approach flights in which the bumble bees finally antennated the floral guide. The portion of approach flights in which the bumble bees antennated the floral guide was low at those artificial flowers which attracted a large number of bumble bees, while it was high at those artificial flowers which attracted a small number of bumble bees. The absolute number of approach flights in which the bumble bees antennated the floral guide was highest at those artificial flowers which attracted an intermediate number of bumble bees, while it was lower at those artificial flowers which attracted a large number of bumble bees and at those which attracted a small number of bumble bees.

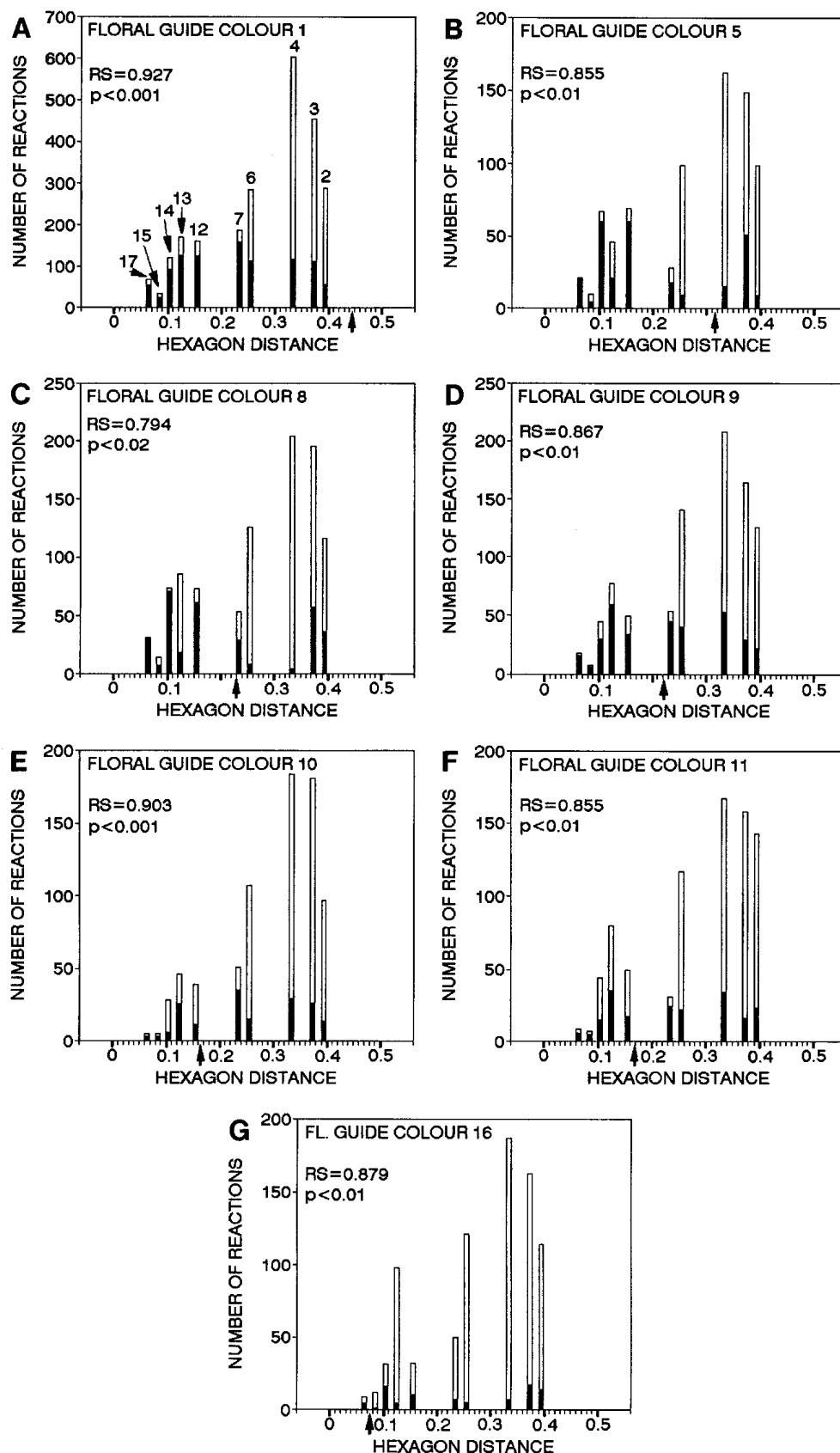
The bumble bees chose among the various corolla colours of the artificial flowers during the approach flight, and they chose among the corolla colour and floral guide colour of each artificial flower during the antennal reaction. The results show that the bumble bees exhibit innate colour preferences during the approach flights and during the antennal reactions.

In the 7 tests, in each of which the same set of corolla colours was offered with a different floral guide colour,

a significant correlation between the frequency of approach flights and the spectral purity of the corolla colours was found (see Fig. 4). The correlation analysis for the antennal reactions is shown for the data presented in Fig. 5.

Although a significant correlation (Spearman rank correlation coefficient $RS = 0.500$, $P < 0.001$) was found between the percentage of cases in which the approach flight towards artificial flowers was terminated by the antennal contact with the floral guide and the spectral purity of the floral guides (Fig. 5A), it is not very convincing (see also partial regression analysis, below). Is the percentage of cases in which the approach flight is terminated by contacting the floral guide with the antennae a function of the colour difference between corolla and guide colour? The correlation between these two parameters is significant ($RS = 0.295$, $P < 0.02$) (Fig. 5B). However, the data points show two trends, one of which corresponds to values of hexagon distances > 0.4 and to percentages of cases in which the approach flight towards an artificial flower was concluded with the antennal contact with the floral guide < 40%. This group of data represents those 14 artificial flowers where both the corolla colour and the floral guide colour have a high spectral purity, but belong to different colour categories (bee-green vs. bee-UV-blue or bee-blue-green). In these cases, the spectral purity of both colours was similar, and the colour difference between the corolla and the floral guide colour was very high. Thus, colour difference between corolla and guide obviously plays some role, but it is clearly not the only parameter which determines whether an approach flight towards an artificial flower ends with the antennation of the floral guide. The strongest correlation ($RS = 0.740$, $P < 0.001$) was found between the percentage of cases in which the approach flights towards artificial flowers were terminated by the antennal contact with the floral guide, and the hexagon distance between the floral guide and the background colour *minus* that between the corolla colour and the background colour of each artificial flower (Fig. 5C). High percentages of antennal contact with the floral guide were found for those artificial flowers offering a floral guide of higher spectral purity than the corolla. The particularly good correlation between choice values and the latter parameter indicates that this is indeed a likely parameter used by the bumble bees. Since the other parameters however, also show significant correlation coefficients, we cannot with certainty exclude any of these, nor rule out the possibility that they all contribute in combination to the choices of the bumble bees. Worse still, the parameters are likely intercorrelated; thus it is possible that correlation of choices with one or two of the parameters tested is simply a by-product of their intercorrelation with the third. To cope with this problem, we performed a standard partial regression analysis on the ranks, that is, we recalculated the 3 correlation

Fig. 4A–G Innate choice behaviour in *Bombus terrestris* at artificial flowers, as a function of the hexagon distance between corolla and background. **A–G** 7 tests in each of which the same set of corolla colours was offered with a different floral guide colour. The numbers serve identification of corolla and floral guide colours as shown in Fig. 2. White columns: frequency of approach flights towards the artificial flowers; dark columns: frequency of approach flights towards the flowers in which the bumble bees finally antennated the floral guide. Small arrows on the abscissa indicate the spectral purity of the guide colour. The Spearman rank correlation coefficient RS for the correlation between the frequency of approach flights and the hexagon distance of the corolla colour to the background is labelled for each test. For comparison, see Fig. 5A in which the percentage of cases in which the approach flight towards an artificial flower was followed by an antennal contact with the floral guide is plotted against the spectral purity of the floral guide colour. The A shows the numbers of the corolla colours according to their ranks in spectral purity



coefficients after eliminating the influence of the respective other parameters. We found the following partial correlation coefficients for the predictor variables:

hexagon distance between the floral guide colour and the background colour $RS_{\text{part}} = 0.482$ ($P < 0.001$), hexagon distance between the floral guide colour and

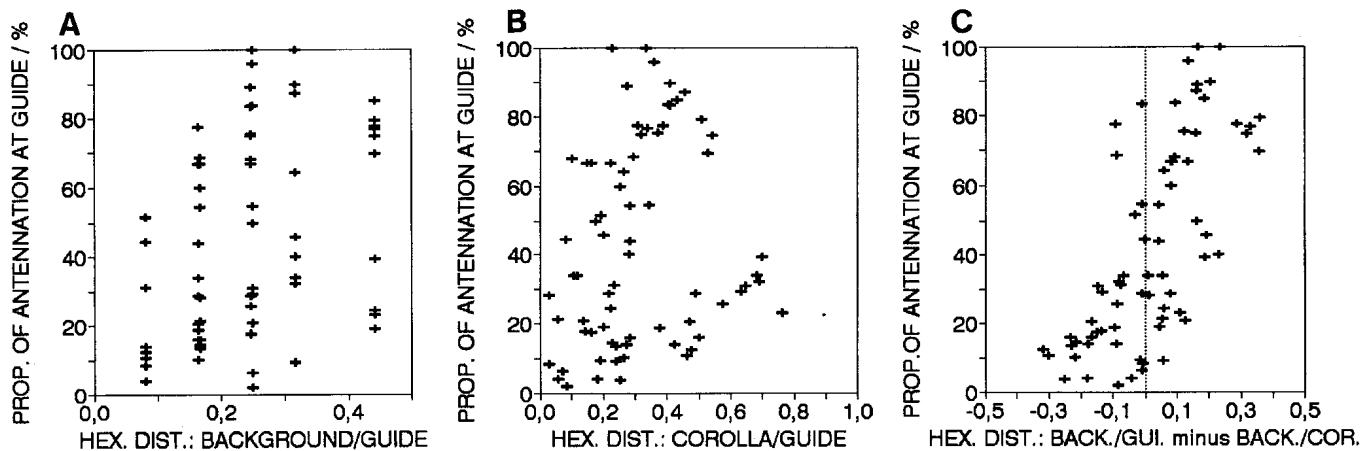


Fig. 5A–C The percentage of cases in which the approach flight towards an artificial flower was followed by an antennal contact with the floral guide is given for all 70 combinations of guide and corolla colours. 7262 approaches were evaluated. Proportion of antennation at the guide as a function of A) the hexagon distance between the background and the floral guide colour; B) the hexagon distance between the corolla and the floral guide colour; and C) the hexagon distance between the background and the floral guide colour minus that between the background and the corolla colour

the corolla colour $RS_{\text{part}} = -0.337$ ($P < 0.01$), and hexagon distance between the floral guide and the background colour minus that between the corolla colour and the background colour $RS_{\text{part}} = 0.745$ ($P < 0.001$). The partial correlation coefficients are very similar to the normal correlation coefficients. Thus, the latter parameter—hexagon distance between the floral guide and the background colour minus that between the corolla colour and the background colour—remains the most likely parameter determining the bumble bees choice behaviour. However, we cannot yet exclude the other parameters with certainty.

Experiment 2

When the same set of two artificial flowers, comprising a yellow (bee-green) and a blue (bee-UV-blue) corolla was presented on different background colours, the frequency of approach flights towards the artificial flowers was dependent on the background colour used (Fig. 6). The distributions of the number of approach flights towards the two artificial flowers combined with the green and with the light blue background differ on the $P < 0.001$ level from the distribution of the number of approach flights towards the two artificial flowers combined with the white background (Chi-square-2*2 test). In consideration of the “room adaptation” assumption (see Fig. 3), the bumble bees approached bee-green corollae significantly more often when they

were presented against a light blue background than when they were presented on the white background or on the green background. The lowest frequency in approaches towards the bee-green colour occurs when the colour difference to the background is the lowest, which is the case with the green background. Likewise, the bees chose the bee-UV-blue corolla most frequently when it was presented against a green background. This colour was approached less frequently when the background was light blue, and thus the colour difference to the corolla was lower.

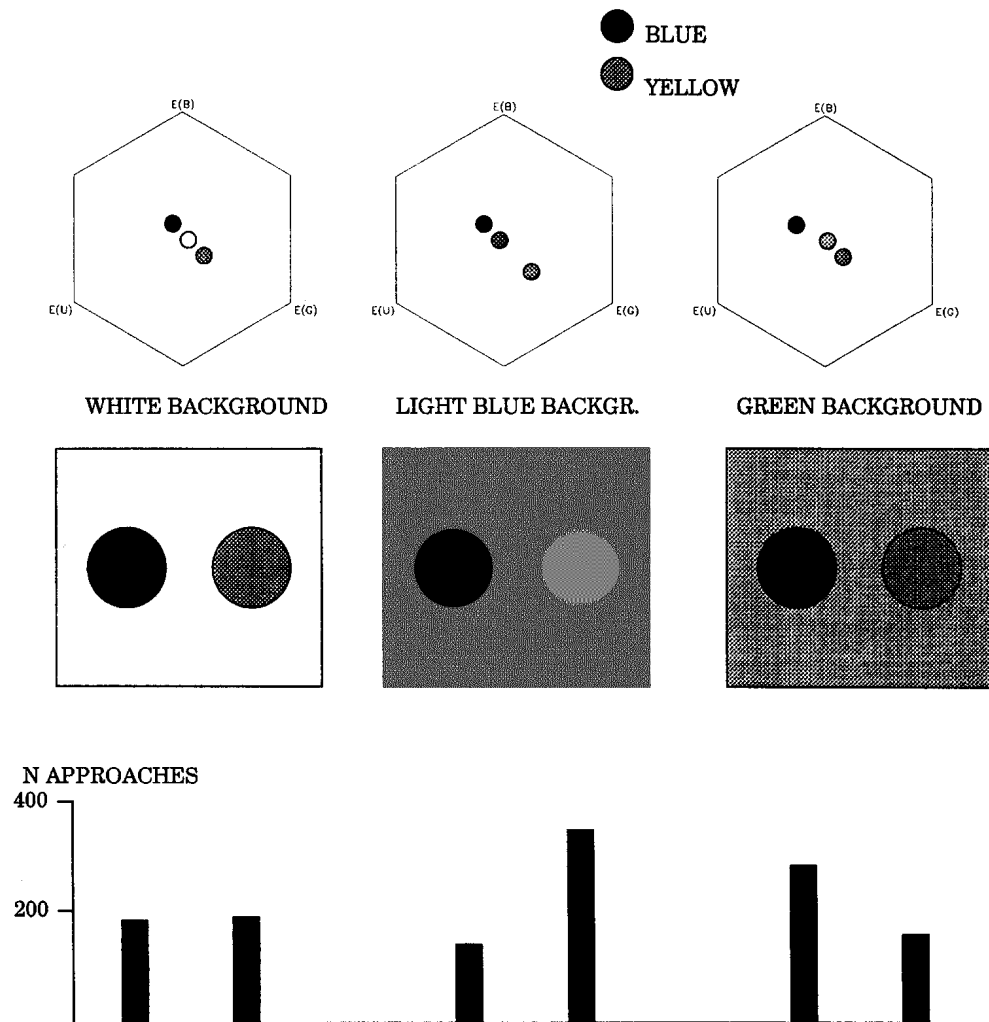
In consideration of the “table adaptation” assumption, the background colours shift into the uncoloured point, and the two corolla colour loci are slightly shifted (Fig. 6). The basic observation of the experiment, however, remains the same: the larger the colour difference of the corolla to its background the more frequently this corolla is chosen.

Experiment 3

In this experiment, approach flights were studied with reciprocal colourations of background and corolla, and the antennation behaviour was studied with reciprocal colourations of corolla and floral guide. When each of the three combinations and flower pairs was tested separately (Exp. 3A), 20% of the approaches towards the artificial flowers were interrupted before the bumble bees contacted them. When the bumble bees oriented towards the artificial flowers on the white background, 92% of the reactions were followed by a contact with an artificial flower, whereas 8% were interrupted. In the case of artificial flowers on the green background, of the reactions towards artificial flowers, 19% were interrupted as were 42% of the reactions towards artificial flowers on the yellow background. All of these differences are significant ($P < 0.001$; Chi-square-2*2 test).

The results shown in Fig. 7 involve only approaches that ended in antennal contact with the artificial

Fig. 6 Frequencies of approach flights of *Bombus terrestris* at a set of two artificial flowers, yellow (bee-green) and blue (bee-UV-blue), which were simultaneously offered against different background colours ($n = 1363$). *Top row*: colour loci of the blue and yellow flowers, assuming that the receptors are adapted to the background tables. *Middle row*: experimental setup – the same 2 flowers colours on 3 different backgrounds. *Bottom row*: number of choices



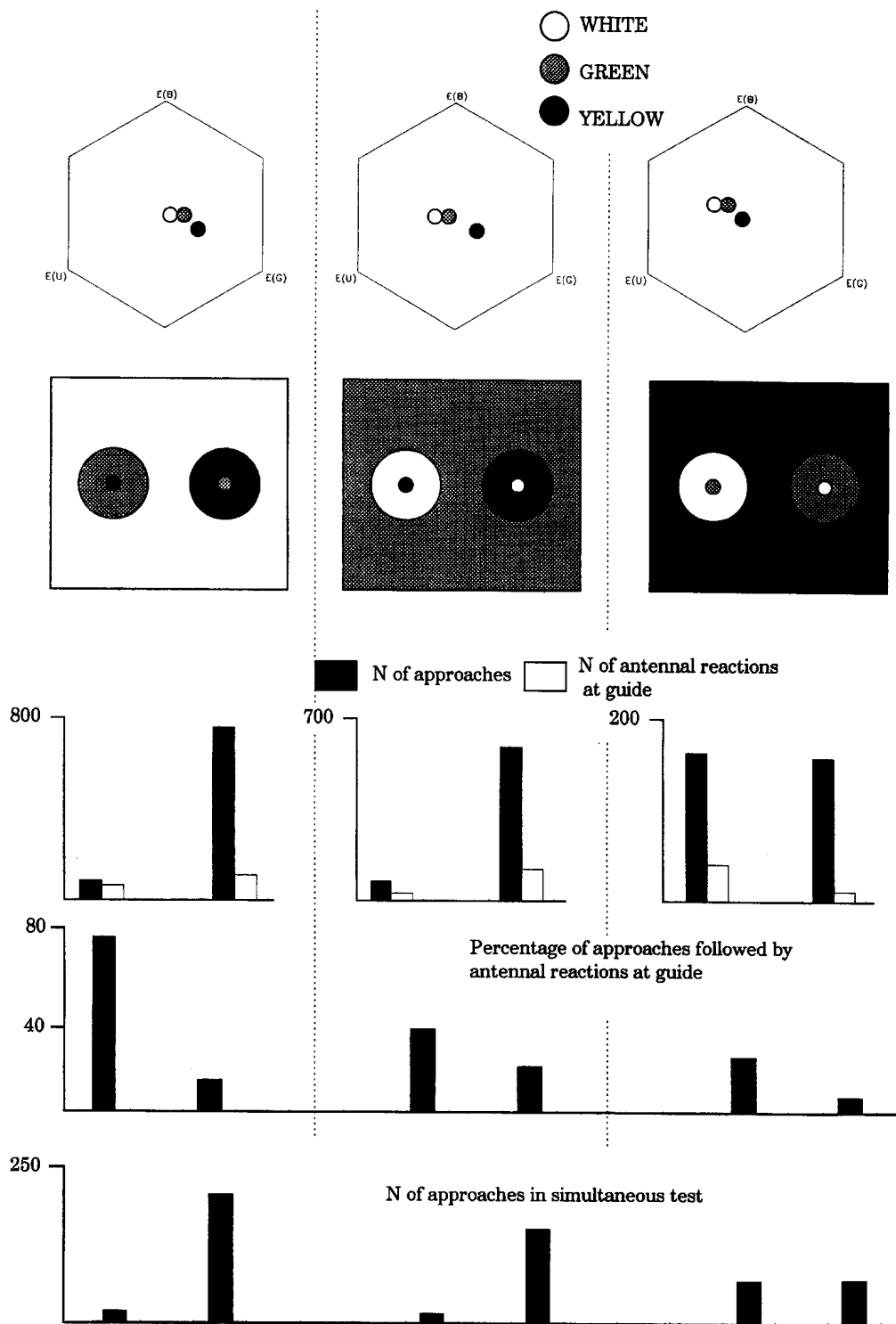
flowers, either with the corolla or with the floral guide. The results are given for the cases, when each of the three combinations and flower pairs was tested separately and when all of the three pairs of colour combinations were offered simultaneously. 1. Separate testing (Exp. 3A): with the white background tables, 90% of the approach flights were directed towards the yellow dummies and 10% towards the green dummies. With the green background, 89% of the approach flights were directed towards the yellow and 11% towards the white dummies. With the yellow background, 49% of the approach flights were directed towards the green and 51% towards the white dummies (Fig. 7). The number of approach flights towards the artificial flowers in the case of the first two pairs of reciprocal colour combinations between background and corolla colour differ significantly ($P < 0.001$; Chi-Square test). 2. Simultaneous testing (Exp. 3B): 39% of the 514 approach flights were directed towards the yellow dummies on a white background and 13% towards the white dummies on a yellow background; 29% towards

the yellow dummies on a green background and 13% towards the green dummies on a yellow background; 4% towards the green dummies on a white background and 3% towards the white dummies on a green background (Fig. 7). The number of approach flights towards the artificial flowers in the case of the first two pairs of reciprocal colour combinations between background and corolla colour differ significantly ($P < 0.001$; Chi-Square test). Thus, the percentages of approach flights up to contact with the artificial flowers chiefly depend on the direction of the colour difference.

The percentage of cases in which the approach flight towards an artificial flower ended with an antennal contact with the floral guide (results of separate testing) was very high at the colour combinations which offered a 3-step gradient of spectral purity ("room adaptation"; see Fig. 3). When a white (bee-uncoloured) background was combined with an artificial flower consisting of a green corolla and a yellow floral guide, the three colours are arranged in a gradient of increasing spectral purity values from peripheral to central parts. 75% of

Fig. 7 Experiments with flower dummies reciprocal in guide and corolla colouration, using three different background colours.

Top row: loci of colours, assuming that the receptors are adapted to the background tables. *Second row:* experimental combinations of the colours shown in top row. *Third row:* total number of choices at corolla and guides (separate testing, Exp. 3A). *Fourth row:* percentage of cases in which the approach flight was followed by an antennal contact with the guide (separate testing, Exp. 3A). *Bottom row:* total number of approach flights during simultaneous testing (Exp. 3B). Only approach flights up to contact with the artificial flowers were evaluated. In the experiment with white background tables 846 choices were evaluated, with green tables 707 choices, and with yellow tables 332 choices. 514 approaches were evaluated for the simultaneous testing



the antennal reactions were directed towards the floral guide if the colours were arranged in a gradient of centripetally increasing spectral purity. This percentage is significantly higher than in all other cases ($P < 0.001$, Chi-Square test). In the case of the reciprocal gradient of spectral purity (yellow background, green corolla, white floral guide), only 7% of the antennal reactions were directed towards the floral guide. In the case of the

4 remaining colour combinations each offering a mixed gradient, the frequency of antennal contacts with the floral guide was between 14% and 36%. In contrast to the results of Exp. 1, the absolute number of approaches terminating in an antennal contact with the guide was higher at combinations of high spectral purity yellow corollae and low spectral purity floral guides than at reciprocal combinations. In this case, the

absolute number of approaches terminating in an antennal contact with the guide was even higher towards artificial flowers with yellow corollae than the *absolute* number of approaches towards artificial flowers with yellow floral guides.

In consideration of the “table adaptation” assumption, the background colours shift into the uncoloured point (Fig. 7). The basic observations of the experiment, however, remain the same: the percentages of approach flights are significantly different for pairs of artificial flowers which were coloured reciprocally with respect to corolla and background. Also with respect to the proportion of antennal contacts, under the assumption of “table adaption”, the basic findings remain: for each of the 3 pairs of artificial flowers which were coloured reciprocally with respect to floral guide and corolla, significant differences in the proportion of antennal contacts with the floral guide were found ($P < 0.01$, Chi-Square test).

Discussion

The experiments demonstrate that naive bumble bee workers (*Bombus terrestris*) exhibit innate colour preferences in two behavioural reactions, i.e. when approaching the artificial flowers from some distance and when selecting an area for the first antennal contact at the colour pattern of the artificial flowers. During the approach flight, the bumble bees chose artificial flowers depending on the corolla colour. During the antennae reaction, they chose between the two coloured areas of the artificial flowers, corolla and floral guide for the first antennal contact with the artificial flower.

Our re-analysis of Lunau's multiple choice test (1990) shows that, using calculated photoreceptor excitations and perceptual colour differences instead of differences calculated from relative quantum flux values to the types of photoreceptors, the innate preferences might indeed be explained by assuming that naive bumble bees rank stimuli according to their spectral purity. This observation could be explained by assuming that bees possess a perception of colour saturation. However, since the more spectrally pure stimuli were also those that contrasted well against the background, we could not exclude the hypothesis that bees performed simple evaluations of colour differences by means of a colour opponent system.

Experiment 2 is critical for testing whether bumble bees *exclusively* evaluate the spectral purity of flower colours. However, the results of Exp. 2 show that the bumble bees make use of the information about the colour difference between the corolla colour and the background during their approach flight. If the corolla colour was constant but its background varied, the attractiveness of the corolla increased when its contrast to the background was large. This result might be

explained when one assumes that the attractiveness of a colour is related to its detectability against a given background. The findings may also be related to Neumeyer's (1980, 1981) quantitative measurements on simultaneous and successive colour contrast in honey bees: the appearance of a particular colour is not only dependent on its own spectral properties, but also on its surroundings and on the ambient adaptation light.

Experiment 3 is critical for testing whether bumble bees *exclusively* evaluate the colour difference between the flower colours. Three reciprocal colourations of background and corolla and of corolla and floral guide were tested. The reciprocal colouration is characterized by a constant magnitude of the colour difference between different floral parts and their background, and at the same time by a reversed direction (sign) of the colour difference. In these tests, the bumble bees exhibited strong preferences for one of the two reciprocally coloured artificial flowers. This result is inconsistent with the hypothesis that the bumble bees' choice behaviour is exclusively determined by the magnitude of colour difference between the artificial flower colours.

Many approaches towards the artificial flowers were aborted before the bumble bees made antennal contact with the flower. The portion of interrupted approach flights was highest if white and green artificial flowers were offered against a yellow background, middle if white and yellow artificial flowers were offered against a green background, and lowest if green and yellow artificial flowers were offered against a white background. Obviously, the frequency of initiated approach flights is dependent on the magnitude of colour difference between the artificial flower colours and the background, which may be related to its detectability. However, whether or not the approach flight is terminated by antennation of the artificial flower is strongly dependent on the direction of the colour difference between background and corolla. Similarly, whether the approach flight is terminated by antennation of the corolla or whether it is terminated by antennation of the floral guide is strongly dependent on the direction of the colour difference between corolla and floral guide. This result is not consistent with the hypothesis that colour difference – as measured by a colour opponent coding system – is the sole explanation for the behavioural data.

Current models of hymenopteran colour perception are based on colour discrimination in trained bees and wasps (Backaus 1991; Chittka et al. 1992). These models predict that distances in colour space should be responded to in terms of their magnitude only, and independent of their directionality. The observations of naive and untrained bumble bees presented cannot be fully explained on the grounds of these models.

What kind of a perceptual parameter might then underlie our observations? A possible explanation is that bees possess a perception of colour saturation comparable to that of humans (Wyszecki and Stiles

1982), as was conjectured by Lunau (1990). Evidence of this assumption is the fact that, although naive bumble bees responded to the magnitude of colour difference, they responded differently to the two directions (signs) of colour contrast (under both adaptation hypotheses). Additional support for the notion that spectrally pure colours have a different salience from colours with low spectral purity comes from honey bee colour learning (Daumer 1956; Menzel 1967; Giurfa et al. 1995). These authors found that bees can be more easily trained to monochromatic lights than to white light or mixtures of white and monochromatic lights. Another possible explanation is that bumble bees judged stimuli according to the unique perceptual dimension of "green-ness", possibly as a component of a more general system of hue perception as known in humans. The present data do not enable us to distinguish between these hypotheses.

The vast majority of bee species visits a wide range of flower species, particularly where nectar foraging is concerned (Michener 1979; Feinsinger 1983; Westrich 1989). This is not surprising when one considers that flower-visitors maximize foraging efficiency in order to maximize numbers of viable offspring (Pyke 1984). The emergence of flower-visitors and the blooming time of particular species may not be reliably synchronized, the reward situation in different flower species is dependant on foraging activities of many visitor species and there are no flower signals that advertise a given reward level with complete reliability (Heinrich 1976; Giurfa et al. 1995). Thus, flower-visitors with high energy requirements, such as bumble bees, should not be confined by means of innate preferences to only a narrow range of flower signals (Heinrich 1979). These bees have a strong capacity for matching their visitation frequencies to floral rewards associated with signals of arbitrary colouration (Heinrich 1976, 1979; Pyke 1984; Greggers and Menzel 1993). However, to facilitate the first encounters of newly-emerged bees with flowers, it is essential to have a generalized innate mechanism that covers the signals of all potential floral food sources, and excludes objects of low foraging interest (Lunau 1990, 1991a,b, 1992, 1993).

Floral signals include a large variety of colours and colour patterns, but they have one general feature in common: the great majority of floral colours has a larger spectral purity than several natural background materials such as green foliage, stones, sand, and autumn leaves (Lunau 1992, 1993; Menzel and Shmida 1993; Endler 1993; Chittka et al. 1994). We have shown here that bumble bees have a generalized inborn mechanism enabling them to distinguish between objects of these two classes, flowers and background materials. Furthermore, the spectral purity of pollen, pollen guides and nectar guides is in most cases higher than that of natural backgrounds (Lunau 1992, 1993; Menzel and Shmida 1993) and thus the observed preferences of the bees are likewise adaptive.

Finally, it is interesting to speculate on how an optimal floral signal should be designed for attracting naive bumble bees. For this purpose, the optimally designed flower will attract a large *absolute* number of bees from a distance and will guide a high *percentage* of approaching bees to antennal contact with the floral guide, since the antennal contact obligatorily precedes landing and because chemical flower signals of the area touched with the antennae promote landing (Lunau 1991a,b). The absolute number of antennal contacts generated through this 2-step process is thus a correlation of how many naive bees will land on a flower. Our results clearly show that it is not possible to simultaneously optimize both steps. *High* spectral purity values are required for a corolla attracting large numbers of bees from a distance, whereas *low* corolla purities combined with high floral guide purities will incite a high percentage of approaching naive bees to inspect the flower further. It is difficult to predict what the optimal tradeoff will be in this intricate situation. However, one has to bear in mind that bee preferences have not evolved for the benefits of plant reproductive success. From the naive bee's point of view, the described preferences are certainly well suited to facilitate the first encounters with flowers.

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