ORIGINAL PAPER

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Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (*Bombus terrestris*) as a case study

Abstract Individual bumblebees were trained to choose between rewarded target flowers and non-rewarded distractor flowers in a controlled illumination laboratory. Bees learnt to discriminate similar colours, but with smaller colour distances the frequency of errors increased. This indicates that pollen transfer might occur between flowers with similar colours, even if these colours are distinguishable. The effect of similar colours on reducing foraging accuracy of bees is evident for colour distances high above discrimination threshold, which explains previous field observations showing that bees do not exhibit complete flower constancy unless flower colour between species is distinct. Bees tested in spectrally different illumination conditions experienced a significant decrease in their ability to discriminate between similar colours. The extent to which this happens differs in different areas of colour space, which is consistent with a von Kries-type model of colour constancy. We find that it would be beneficial for plant species to have highly distinctive colour signals to overcome limitations on the bees performance in reliably judging differences between similar colours. An exception to this finding was flowers that varied in shape, in which case bees used this cue to compensate for inaccuracies of colour vision.

Keywords Colour constancy · Colour vision · Evolution · Flowers · Insect vision

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Introduction

Bumblebees (*Bombus terrestris*) have trichromatic colour vision with photoreceptors maximally sensitive at about 350, 440 and 540 nm (Fig. 1A) (Menzel and Backhaus 1991; Peitsch et al. 1992), and bees use their colour vision to find rewarding flowers (Chittka et al 1997). In a natural environment coloured flowers are often encountered one at a time, especially when one takes into account the limited resolution of compound eyes (Land 1997; Spaethe et al. 2001; Spaethe and Chittka 2003). This means that the bee's visual system is often required to make discriminations when flowers are viewed successively, and it is important to know how well bees can perform this task when flowers are more or less similar in colour.

Bees often do not visit different flowers at random, but exhibit flower constancy and restrict visits to a single species for a period of time (Waser 1986; Chittka et al. 1999). Flower constancy has considerable reproductive benefits for plants (Chittka et al. 1999), and individual bees appear to benefit due to limitations on their memory organisation and retrieving processes for handling multiple food sources (Greggers and Menzel 1993; Chittka and Menzel 1992; Chittka et al. 1999). Flower colour is an important determinant in flower constancy (Waser 1986; Chittka and Menzel 1992; Chittka et al. 1997), and the requirement of producing visual signals that can uniquely identify flower species is often considered to be the driving force that has led to the diversity of flower colours in nature (Chittka and Menzel 1992; Kevan and Backhaus 1998). However, Vorobyev and Menzel (1999) produced a theoretical analysis based upon computational modelling of a bee's ability to discriminate flowers and deduced that there is little evolutionary pressure for plants to diverge in colour signal. They argued that the ability of insects to discriminate the colour signals of different flowers was fine enough that there was little chance of bees mistaking the colour signals of different flowers. However, this conclusion does not fit with field
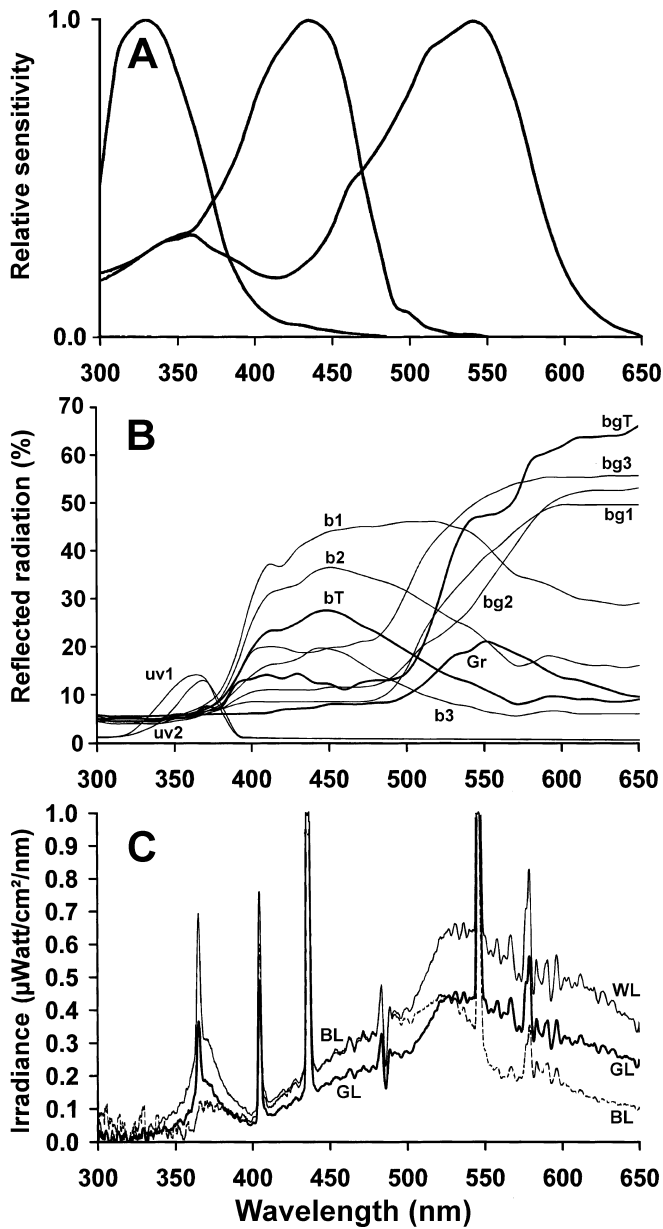
Fig. 1A–C Data used to calculate colour loci of the stimuli used in experiments. **A** The relative spectral sensitivity of bumblebee (*Bombus terrestris*) photoreceptors normalised to a maximum of 1.0 (data from Peitsch et al. 1992). **B** Spectral reflectance functions of stimuli. *uv1, uv2* ultraviolet colours; *bT* blue target flower; *b1–3* similar distractors; *Gr* painted green background; *bgT* blue-green target flower; *bg1–3* similar distractors. **C** Spectral distributions of the three illumination conditions. *WL* white light; *GL* grey light; *BL* blue light
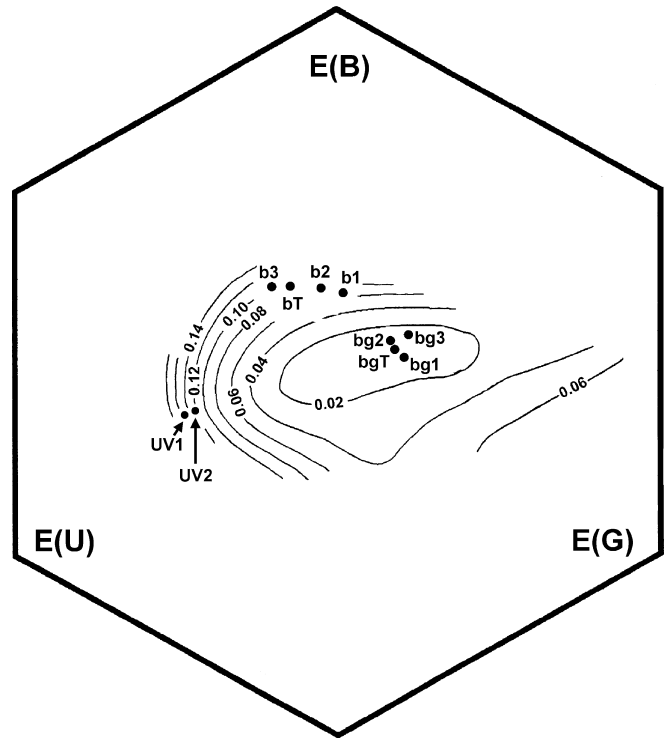

Fig. 2 The loci of coloured stimuli (see Fig. 1) that were used in experiments to evaluate how spectrally variable illumination affects choices between similar colours. Loci are plotted in a hexagon colour space (Chittka 1992) considering grey light illumination. A contour map of predicted colour shift considering von Kries colour constancy is superimposed over the colour loci (Dyer 1998, 1999b)

observations that bees generalise to flowers of similar colouration (Chittka et al. 1997; Gumbert 2000), and that high levels of flower constancy are only observed when flowers are of pronounced colour difference (Chittka et al. 2001). Colour discrimination means that a visual system is capable of correctly choosing between stimuli. In contrast, generalisation is a failure to discriminate between stimuli even though a visual system is theoretically capable of doing so. We hypothesise that

this apparent contradiction in discrimination ability is likely to become apparent when stimuli are similar in their appearance, and are viewed successively. This question is important to understand how and why plants have evolved colour signals in order to maximise the probability of reliable identification by bees.

The difficulty posed by flowers being similar in colours is also relevant to another task that the visual system of the bee is required to solve. When foraging in a field the spectral composition of illumination may vary due to the presence of sunlight, clouds, shade and/or filtering by foliage (Lythgoe 1979; Dyer 1998). The radiation reflected from a flower is the product of both its spectral reflectance properties and the spectral quality of the illumination; however, the signal that is biologically relevant to a bee is the flower's reflectance properties. The bee's visual system must therefore have a mechanism to compensate for differences in the spectral quality of illumination or the benefits of having colour vision would be greatly compromised (Dyer 1998).

The phenomenon of colour constancy has been demonstrated in honeybees with a variety of behavioural experiments (Mazokhin-Porshnyakov 1966; Neumeyer 1981; Werner et al. 1998). However, considering a chromatic adaptation explanation and applying a von Kries model of colour constancy, Dyer (1998, 1999a, 1999b) showed that the bee's photoreceptors are not

predicted to be able to provide perfect colour constancy (Fig. 2). It was predicted that the degree of correction is different for different regions of bee-colour space, and this finding correlated with the findings of Chittka et al. (1994) that there is not an even distribution of flower colours distributed in a hexagon colour space (Dyer 1998; Kevan et al. 2001). Could it be that some plants avoid using short-wavelength rich colours because such flowers will be more readily confused with others under conditions of changing illumination? This is important for the understanding of flower evolution because there is good evidence that many plants have evolved flower colours to suit the visual system of bees (Chittka and Menzel 1992; Chittka 1996; Gumbert et al. 1999).

In this study we test whether the similarity of distractor colours presented to bees may limit their ability to correctly choose target flowers. We also address the question of specifically testing if colour constancy is approximate in bees, and potentially variable for different regions of colour space as a von Kries model predicts (Dyer 1998). Finally, we test whether flowers that are similar in colouration might benefit from diverging in floral shape.

Materials and methods

Foraging environment

Individually marked bumblebees (*Bombus terrestris*) were trained in a flight arena (120×100×35 cm) fitted with a UV-transparent Plexiglas cover. The arena floor was painted green to match the spectral reflectance of foliage (Fig. 1B). Illumination for the experiments was provided by six DURO-TEST 40-W True-Lite tubes and one OSRAM 36-W Blacklight tube. Tube flicker was converted to 1,200 Hz using special ballasts (Osram Quicktronic QT-Eco 1×58/230–240) and light diffused by a single sheet of Rosco 216 UV-transmitting white diffusion screen (Rosco, Germany) to provide an even and homogenous source. Three types of illumination were used: (1) unfiltered; (2) filtered by one sheet of Arri 400.15ND (a neutral-density filter that evenly transmits 70% of light from 300 to 650 nm); or (3) two sheets of Rosco (Germany) 061 blue filter (this UV-transmitting filter simulates a blue skylight foraging environment). These three illumination sources were defined as white light, grey light and blue light, respectively. Spectral irradiance of the three sources was measured with an Ocean Optics (Dunedin, Fla., USA) spectrometer (S2000) relative to a calibrated deuterium/halogen radiation source (DH-2000-CAL; Fig. 1C). The grey light and blue light sources were matched so that there was an equivalent number of photons from 300 to 650 nm. This allowed for a change in the spectral quality of illumination whilst keeping illumination brightness constant.

Bumblebees were housed in a two-chamber wooden nesting box (28×16×11 cm) connected to the flight arena with a transparent Plexiglas tube. For 5 days before the experiments bees collected 2 mol l^{-1} sugar water from a transparent glass feeder located at random arena positions. Pollen grains were provided directly to the nesting box. Foragers were captured at the glass feeder and individually marked with a small plastic number on the thorax. Environment familiarization was in white light.

Stimuli

Artificial flowers were either plastic disks (diameter 26 mm, thickness 4 mm) with a small hole in the centre to hold fluid (diameter 4 mm; depth 2.5 mm) or Schott UG1 filters (diameter 26 mm; centre hole 4 mm) placed above sand-blasted aluminium disks. Plastic disks were painted with a variety of Revell (Germany) matt paints. Two types of artificial UV flowers were made by placing either Lee 035 (Lee Filters, USA) or Lee 103 filters between the Schott UG1 filter and the aluminium disk. As the aluminium disk reflects the radiation passing through the filters this produces UV-reflecting flowers. Spectral reflectance of stimuli was measured with a Varian DMS100 reflectance spectrophotometer calibrated against a Varian polytetrafluoroethylene standard.

Calculation of colour distance for bumblebees

Colour loci of stimuli were calculated in a hexagon space (Chittka 1992) considering the spectral sensitivity functions for bumblebee photoreceptors (Menzel and Backhaus 1991; Peitsch et al. 1992).

The relative amount of light absorbed by each photoreceptor class is given by P:

$$P = R \int_{300}^{650} Si(\lambda)/(\lambda)D(\lambda)d\lambda \tag{1}$$

where $Si(\lambda)$ is the spectral sensitivity of the (UV, blue, green) receptor class, $I(\lambda)$ is the spectral reflectance function of the stimulus, $D(\lambda)$ is the spectral distribution of the illuminant and $d\lambda$ is the wavelength step size. Spectral irradiance was converted to relative photon flux for the calculation and the visual system was modelled to be adapted to the green background.

The variable R is the adaptation coefficient, which depends on the background stimulus (I_B):

$$R = 1 \left/ \int_{300}^{650} S(\lambda)/_B(\lambda)D(\lambda)d\lambda \right. \tag{2}$$

The transduction of photoreceptor absorption (P) into receptor excitations (E) is given by:

$$E = Pl(P + 1) \tag{3}$$

Coding is performed by two unspecified colour opponent mechanisms and colour distance can be calculated as the Euclidean distance between stimuli loci in colour space (Chittka 1992). Figure 2 shows the colour loci of stimuli.

Training conditions

Movement of individual bees from the nesting box to the arena was controlled with vertically operating shutters. In the arena a marked bee was given a single bout with five target flowers filled with 20 μl of sugar water. This ensured the first flowers visited were rewarding. In subsequent training bouts five distractor flowers were also presented in the arena. The distractor flowers contained either 20 μl water (experiment 1) or a bitter punishment of 20 μl of 0.12% quinine hemisulphate salt in water (experiments 2 and 3). The quinine hemisulphate salt solution is scentless and has an aversive taste for insects (Fresquet et al. 1998). The spatial arrangement of flowers was randomised each bout. A bee collected sugar water from target flowers until satiated, at which point it returned to the nesting box. During each bout visits to target and distractor flowers were scored (where a bee clearly made contact with a flower). To remove possible olfactory cues, flowers were washed in 30% alcohol at the end of each bout. At the conclusion of every third bout the arena floor was washed with 30% alcohol (Spaethe et al. 2001).

At the end of the training procedure the frequency with which a bee could distinguish between flowers was determined in a single non-rewarded bout to exclude olfaction or position learning. Bees show an improvement during training (Giurfa et al. 2001) and we used the following preconditions to establish when a bee had learned to discriminate colours. For an individual bee to be tested in a non-rewarded bout it had to have either two consecutive rewarded bouts where it exclusively landed only on target flowers, or achieved five consecutive rewarded bouts where the frequency of

visits to the target flower was greater than 50% in each bout. If one of these preconditions had not been met by the completion of the 12th training bout, the bee was tested with a non rewarded bout.

Experiment 1: How colour distance between rewarding and non-rewarding flowers affects discrimination

Sequential testing of large colour distances (0.185, 0.102 and 0.062 hexagon units)

The ability of bees to correctly choose blue target flowers when presented amongst distractor flowers of similar colour was tested. White light illumination was used for training and testing. The target and distractor flowers were blue for human vision, having a maximum reflectance between 400 and 500 nm (Fig. 1B). The target flower (flower bT) colour was always the same and different colour distances were achieved by varying which type of distractor flowers (b1–3) were presented in the arena. Target flowers were filled with 20 μl of sugar water and distractor flowers were filled with plain water. The three largest colour distances (0.185, 0.102, 0.062) were tested sequentially with six bees.

Testing of small colour distances of 0.027 and 0.045 hexagon units

The small colour distances of 0.027 and 0.045 hexagon units were tested separately. White light illumination was used for training and testing. A separate group of seven bees was tested on the colour distance of 0.027 hexagon units and a separate group of five bees on the colour distance of 0.045 hexagon units.

Testing of colour distance 0.062 hexagon units under variable level of illumination

A separate group of six bees were tested on the colour distance of 0.062 hexagon units. Discrimination in white light was evaluated with non rewarded tests, and each bee was then trained again to meet a precondition to ensure motivation. Each bee was then tested in grey light to determine whether continued training after the first non-rewarded test or a change in illumination intensity (but not spectral content) might affect the bee's ability to discriminate between similar flower colours.

Experiment 2: How spectrally variable illumination affects discrimination ability for different regions of colour space

This experiment tested the ability of bees to discriminate between similar colours when there was a change in the spectral quality of illumination. The first illumination condition used was grey light representing the spectral quality of daylight, and the second illumination condition used was blue light representing short-wavelength-rich skylight. The absolute number of photons available for vision in these two illumination conditions was equivalent over the spectral range of 300–650 nm, so that the change in illumination conditions was one of spectral content rather than illumination level.

Punishing bees for incorrect choices leads to an increase in discrimination ability (Chittka et al. 2003). In experiment 2, the target flowers were rewarded with 2 mol l^{-1} sugar water and the distractor flowers contained quinine hemisulphate. This training ensured that bee motivation to perform the task accurately was high.

Blue stimuli

The colour distances of 0.185, 0.102 and 0.062 hexagon units for each of six bees were sequentially tested. After discrimination ability in grey light had been determined with non-rewarded tests each bee received additional rewarded bouts to ensure a high level of motivation. After this training each bee's ability to discriminate flowers when there was a change in the spectral quality of illumination to blue light was determined in a non-rewarded test.

Blue-green stimuli

The colour distances of 0.070, 0.040 and 0.029 hexagon units were sequentially tested with six bees. After discrimination ability in grey light had been determined with non-rewarded tests, each bee received additional rewarded bouts to ensure a high level of motivation. After this training procedure, the bees' ability to discriminate flowers when there was a change in the spectral quality of illumination to blue light was determined in a non-rewarded test.

Ultraviolet stimuli

Six bees were trained to discriminate between UV-reflecting colours. Due to the limited possibilities available to produce UV-reflecting stimuli that have reflectance curves approximating those of real flowers we were only able to test one pair of stimuli with a colour distance of 0.035 hexagon units. The ability of the bees to discriminate between this colour pair was so poor in grey light that we did not evaluate the bees' discrimination in blue light (see Results).

Experiment 3: Do bees use flower shape as an alternative cue to compensate for inaccuracies in colour discrimination tasks?

The ability to discriminate between similar blue colours (0.062 hexagon units) when there was also a difference in the flower shape was tested with a separate group of five bees. On each distractor flower a four section radial pattern was painted with Revell matt black paint, whilst the target flower had no pattern. Bees prefer radial patterns (Lehrer et al. 1995; see their Fig. 6a for an example of the shape used here) and the pattern was placed on the distractor flower so that innate preferences would not help the bee solve the visual task of finding the target flowers. Each bee was trained and tested in grey light illumination and then re-tested in blue light illumination using the preconditions described above.

Results

Experiment 1: How colour distance between rewarding and non-rewarding flowers affects discrimination

Bees were able to learn to discriminate between very similar colours (Fig. 3A). There was no statistical difference between sequential or single testing for the colour distance of 0.062 hexagon units (Mann Whitney U-test: $U = 9.0$; $n = 6 + 6$; $P = 0.150$, NS) and thus results for this colour distance were pooled. Figure 3A shows that for stimuli that are more similar in colour, there is a decrease in the frequency of correct choices made by bees when the stimuli are viewed successively.

In addition to similar colours being more difficult for bees to discriminate, bees took longer to learn to solve the problem. Figure 3B shows the rate at which bees learnt to discriminate large, intermediate and small colour distances. For the largest colour distance tested, the bees almost immediately learnt to only visit the rewarding target colour. Indeed, the initial rewarded bout where only the target colour was presented for a
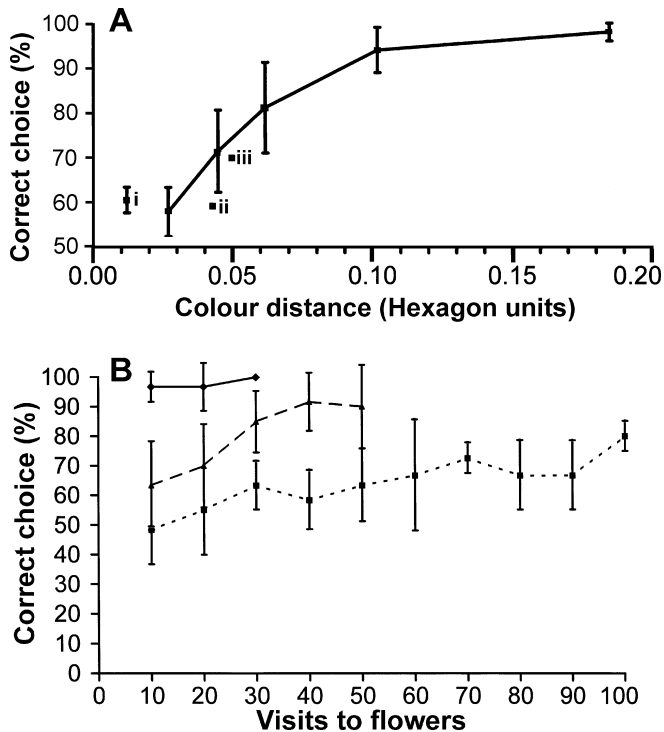
Fig. 3A,B The ability of bumblebees to discriminate between stimuli depending upon the similarity of the target colour to distractor colour (mean ± SD). Stimuli were 26-mm-diameter painted discs presented in a flight arena with controlled illumination. **A** The ability to correctly choose the target colour decreases with a reduced colour distance between target and distracter flowers, even for colour distances high above discrimination threshold. We also show data points from three separate studies: (1) data from Chittka et al. (2003) for non-punished condition where the result was significant from chance ($X^2 = 8.73$, $df = 1$, $P < 0.05$) and colour distance was 0.012 hexagon units, (2) Lehrer (1999) and (3) Thery and Casas (2002) based on the data of von Helversen (1972b). **B** The number of visits it took bumblebees to learn to discriminate a target colour depending upon colour similarity of the distractor colour. To encourage motivation, bees were initially given a single foraging bout in which only the rewarded target colour was presented. For large colour distances (0.185 hexagon units; *continuous line*) the initial experience with the target colour was sufficient to immediately promote a high level of discrimination. For an intermediate colour distance (0.102 hexagon units; *broken line*) it took bees more visits to learn to reliably discriminate between stimuli. For a small colour distance (0.062 hexagon units; *dotted line*) it takes a large number of visits to learn to discriminate between stimuli reliably

single bout (see Materials and methods) was effective in training the bee to avoid distractor flowers that were separately by a relatively large colour distance. For the intermediate colour distance, bees quickly learnt to discriminate at levels greater than 70%, whilst for a small colour distance it took a very long time to learn to make reliable discriminations at greater than 70% frequency of correct choice (Fig. 3B). Figure 3 shows that the probability of bees mistaking the colour of a flower depends upon the similarity of targets and distracters. Colours that are very similar may be distinguishable at the receptor level of processing when viewed simultaneously, but when viewing is successive more errors

occur when the distracter colour is more similar to the target colour.

For the six bees that were trained and tested only on the colour distance of 0.062 hexagon units in white light, we also used a within-subject comparison to evaluate whether their choice frequency was affected when retested in grey light (see Materials and methods). These two illumination conditions have very similar spectral characteristics and represent a change in illumination intensity. In white light the bees' frequency of correct choice was 76.5 ± 12.2 (mean ± SD) and in grey light the frequency of correct choice was 79.4 ± 12.7. Testing the bees in these two illumination conditions was not statistically significant (Wilcoxon signed ranks test; $Z = -0.734$; $P = 0.463$; two tailed test, NS) showing that continuing to train bees after a non-rewarded test does not affect their ability to discriminate between colours. The non-significant result also shows that a 30% variation in illumination level did not affect a bee's discrimination ability. This result shows that the training conditions lead to a consistent performance level when bee discrimination is evaluated on two separate occasions under spectrally equivalent illumination sources.

Experiment 2: How spectrally variable illumination affects discrimination ability for different regions of colour space

Blue stimuli

For the colour distances of (0.185, 0.102 and 0.062 hexagon units) the ability of six bees to discriminate between target and distractor flowers was evaluated in non-rewarded tests in grey light. Immediately after this non-rewarded test, each bee was retrained to ensure continued motivation to forage. Each bee's ability to discriminate between the flowers was then re-evaluated in blue light in a non-rewarded test. Modelling of this illumination change in bee colour space shows that the colour loci of blue stimuli are expected to have a relatively large colour shift assuming a von Kries constancy mechanism (Fig. 4A). With the change in spectral illumination conditions, there was a significant decrease (see section on blue-green stimuli below for statistics) in the frequency of correct choices to the target flowers (Fig. 4B).

Blue-green stimuli

For a change in illumination colour, the loci of blue-green stimuli are expected to have a relatively small colour shift considering a von Kries constancy mechanism (Fig. 4C). For the colour distances of 0.070, 0.040 and 0.029 hexagon units a separate group of six bees were tested in grey light and then in blue light (Fig. 4D). To compare the ability of the bees to solve the task of correctly choosing target flowers from the blue and blue-green regions of hexagon colour space, we used a
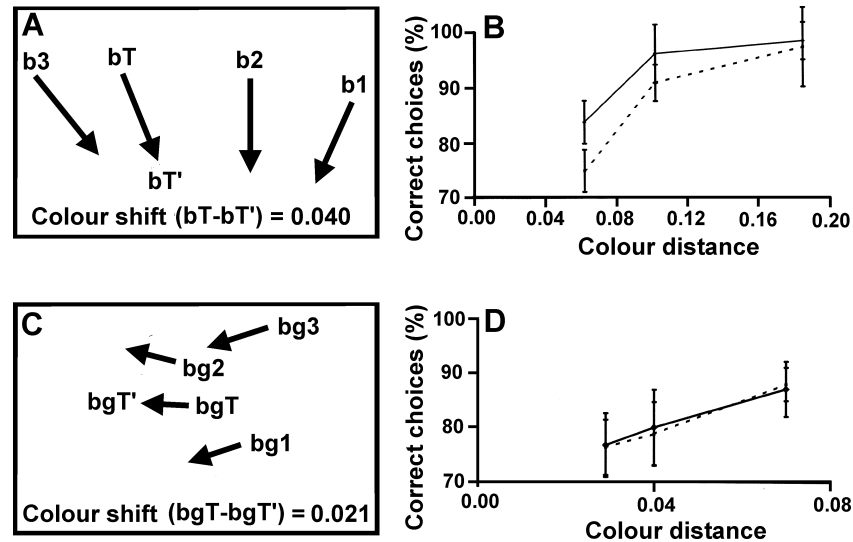
Fig. 4A–D The ability of bumblebees to correctly discriminate target colours in different colours of illumination. **A** The shift in the locus of the blue stimuli in spectrally variable illumination considering von Kries constancy (the height of the box represents 0.1 hexagon units). The *tail of the arrow* is in grey light and the head is in blue light. The blue target flower (*bT*) shifts to position (*bT'*) when the illumination colour changes (colour shift of 0.040 hexagon units). **B** The frequency of correct choices by bumblebees discriminating between similar blue colours in spectrally variable illumination. The *straight line* is in grey light and the *dashed line* is blue light. **C** The shift in the locus of the blue-green stimuli in spectrally variable illumination. **D** The frequency of correct choices between similar blue-green colours in spectrally variable illumination. The ability of bees to make a correction for illumination colour change is relatively poorer for blue colours compared with blue-green colours. This behavioural result is in line with von Kries colour constancy predictions of colour shift (see text for statistics)

two-way mixed ANOVA. In spectrally variable illumination, bees made more errors in choosing the target flower when the illumination colour changed ($ss = 150.5$, $df = 1$, $F = 10.625$, $P < 0.01$), and there were more mistakes for flowers from the blue region of colour space compared to the blue-green region of colour space ($ss = 187.0$, $df = 1$, $F = 6.144$, $P < 0.05$). This shows that bees have only approximate colour constancy and that the ability of bees to make a correction for a change in illumination colour is different for the different regions of bee colour space (Fig. 4B, D). This finding is in agreement with a von Kries type mechanism of colour constancy for bees.

Ultraviolet stimuli

Six bumblebees were trained to discriminate between the UV-reflecting colours. Because of the limited possibilities to produce UV-reflecting stimuli that have reflectance curves approximating real flowers, only a colour distance of 0.035 hexagon units was tested. Discrimination between the stimuli was 59.1 ± 2.5. This result is significantly different from chance ($X^2 = 5.28$, $df = 1$, $P < 0.05$), however, it was clear that the bees found it a

very difficult task and hence we did not evaluate discrimination ability in spectrally changed illumination conditions.

Experiment 3: Flower shape

The ability of five bees to discriminate between similar colours (0.062 hexagon units) when there was also a difference in the flower shape was tested. Bees were trained and tested in grey light, and then re-tested in blue light. In both of these illumination conditions all five bees choose the target flower with 100% accuracy. This compares to a discrimination ability of 76.5 ± 12.2 for similar colours that have no difference in shape, showing that when flowers of different species are similar in colour it would be of benefit to diverge in flower shape to maintain pollinator constancy. Even though bees innately prefer a radial pattern (Lehrer et al. 1995), they quickly learnt to avoid it in this test situation. As the target colour was visited with 100% accuracy in either illumination condition, bees can use shape cues to avoid mistaking similar colours in both constant and spectrally variable illumination conditions.

Discussion

The problem of colour similarity

To understand how flower colours have evolved to maintain pollinator constancy it is important to understand how a bee can process colour information. Based upon computational modelling, Vorobyev and Menzel (1999) argue that a bee has the ability to discriminate the colour signals of different flowers very accurately, and hence there is little chance of bees mistaking the flowers of different species.

However, in humans the ability to accurately judge colour is significantly reduced when stimuli must be

viewed successively rather than simultaneously (Newhall et al. 1957), and the time-course for deterioration of discrimination with successive viewing conditions can be as short as 60 ms (Uchikawa and Ikeda 1981). It is clear from our results that the similarity of a distractor stimulus to a target stimulus has a significant influence on the reliability with which a target colour can be chosen, and the effect is evident for colour distances high above the discrimination threshold (Fig. 3A). This finding is consistent with previous studies that also observed that discrimination improved with greater colour distances between target and distracter flowers (Giurfa et al. 1994, Lehrer 1999). The shape of the discrimination curve we report is not a steep psychometric function, but more closely resembles a shallow sigmoidal-type function (von Helversen 1972a). Our finding that insects experience difficulty when distinguishing between similar colours fits both with ecological findings that flower constancy does not approach high levels unless the colouration of competing flowers is reasonably distinct, and the observations that bees often generalise to similar colours (Chittka et al. 1997; Gumbert 2000). The inability of bees to make as finer colour discriminations as theoretical considerations might suggest (Vorobyev and Menzel 1999) is likely to be due to the fact that the coloured flowers in the flight arena were most often viewed successively, a condition that is often met in nature. Figure 3 supports our hypothesis that when choosing between stimuli presented successively the ability of bees to solve a colour visual problem is limited by the similarity of targets and distracters.

The finding that the ability of bees to discriminate between flowers of similar colour has a shallow sigmoidal-type shape (Fig. 3A) also helps explain why UV-reflecting-white flowers might be rare in nature (Dyer 1996; Kevan et al. 1996). This is possibly because, when viewed against a foliage background UV-reflecting white flowers have a similar colour for bees (about 0.07 hexagon units; Spaethe et al. 2001) and provide poor colour contrast (Spaethe et al. 2001). Spaethe et al. (2001) found that it took bumblebees twice as long to detect UV-reflecting white flowers as opposed to non-UV-reflecting white flowers when stimuli were presented on a green background. For human vision, foliage is perceived as a very distinctive chromatic colour, but this is mainly because the primate medium- and long-wavelength-sensitive receptors appear to have evolved to maximally facilitate detection of targets against a green foliage background (Sumner and Mollon 2000). The spectral reflectance of most foliage has a small variation in reflectance across the visible spectrum of bees (Chittka et al. 1994), especially compared to flowers that often strongly reflect or absorb different wavelengths of radiation (Chittka and Menzel 1992; Chittka et al. 1994). When flowers reflect all wavelengths perceived by bees reasonably equally the colour signal is similar to foliage. The phenomenon of similar colours being more difficult to detect is the basis of camouflage strategies in some animals (Cott 1957), but for flowers that 'want' to

be seen by bees, it is best to avoid being similar to the background stimulus. In a study of target-background similarity on visual search in humans it has also been reported that with decreasing colour distance that there is an increase in reaction time for locating target stimuli (Farmer and Taylor 1980). The explanation for this may be evident in the finding of Komatsu and Ideura (1993), who showed that the discharge rates of single neurons in macaque monkeys (*Macaca fuscata*) is correlated with the relative position of colour similarity of stimuli on a colour map. So while discrimination based upon colour vision may be possible for small colour distances, with larger colour distances the probability of successful detection or discrimination is improved.

The problem of colour constancy

A significant problem bees encounter when identifying a flower by its colour is the spectral quality of illumination is variable (Dyer 1998). Neumeyer (1981) showed that honeybees exhibit colour constancy, but her results also show that honeybees make more errors when there is a larger change in illumination colour (Dyer 1998, 1999b). Dyer (1999b) modelled the training test field locus used by Neumeyer (1981) in a colour hexagon considering a von Kries-type correction mechanism and found that there was a significant linear correlation between choice errors by bees and the predicted colour shift (Fig. 5). This shows that although bees can exhibit colour constancy, they make more errors when the colour shift is greater and suggests that bees have only approximate colour constancy.

We tested whether a change in illumination colour led to a decrease in a bee's ability to choose between similar
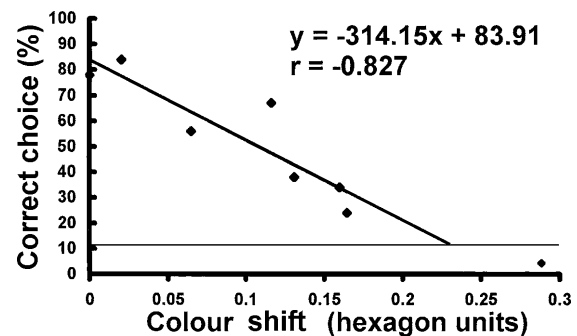

$$y = -314.15x + 83.91$$
$$r = -0.827$$

Fig. 5 Frequency of honeybees correctly choosing a colour patch versus the relative colour shift of the patch when illumination is varied from the initial training source to a test illumination source. For each illumination source, the photoreceptors are weighted to simulate von Kries colour constancy and the colour shift is thus a prediction of imperfect colour constancy. The *horizontal line* is the value where bee foraging was random. The bold line shows the linear regression of the data (Pearson $r = -0.827$; $P < 0.05$ for 1-tailed test) suggesting that the von Kries adaptation explains the bees' poorer performance in spectrally variable illumination. Data derived from the materials and methods of Neumeyer (1981) and plotted in a hexagon colour space (Dyer 1999b) considering the spectral sensitivities of the honeybee (Menzel and Backhaus 1991)

colours, and if the ability to judge colours in spectrally variable illumination was different for different regions of colour space. We found that colour constancy is approximate in bumblebees, and that the correction is poorer for flowers that predominantly reflect more short-wavelength radiation. Dyer (1999a) showed that a von Kries model of colour constancy predicts a poorer solution for flowers in the UV to blue areas of colour space due to the asymmetric spectral shape of bee photoreceptor sensitivity functions. This asymmetry is caused by the absorption of short wavelength radiation by the beta-peaks of the medium and long-wavelength-sensitive photoreceptors (Dyer 1999a), and it was predicted that this could explain the rarity of UV flowers in nature. We were unable to find a combination of artificial UV stimuli that resembled real flowers and that bees distinguished reasonably well (above 70% discrimination), but it was possible to use blue and blue-green flowers to test the theory that colour constancy should be different for the different regions of colour space. Blue flowers are rarer in nature compared to blue-green flowers (Chittka et al. 1994), which is surprising because flower-visiting bees have an innate preference for blue flowers (Giurfa et al. 1995; Chittka et al. 2001). The explanation for the rarity of blue flowers is unlikely to be phylogenetic of biochemical constraints, because there are a very large number of plants that can produce blue coloured flowers (Chittka et al 1994). In spectrally variable illumination, bees were significantly poorer at choosing blue flowers compared with blue-green flowers (Fig. 4); and this result is consistent with the notion that these flowers are relatively rare in nature because they are less easily identifiable when the spectral content of the illumination changes. The results do not necessarily show that colour constancy is perfect for blue-green flowers, but that for the illumination change tested, the bees were better able to make a correction for blue-green flowers compared with blue flowers. This is despite the fact that the blue-green target colour was tested with distractor flower colours whose loci were both very similar and lay at coordinates surrounding the target colour (Fig. 4C). For the blue flowers there is a considerably larger colour shift when illumination changes and the hypothesis that those similar colours might be shifted closer together is confirmed by the significant decrease in correct choices by bees (Fig. 4A and B). This is the first empirical demonstration that colour constancy varies in different areas of colour space, and that this variation is consistent with the assumption that colour constancy is limited by a von Kries type mechanism of photoreceptor adaptation.

Werner et al. (1988) demonstrated colour constancy in honeybees using a colour trans-illuminated Mondrian display. In a test stage, the illumination conditions were changed so that one of the alternative squares emitted the same radiation fluxes as the training square had in the training condition. In the test condition bees still preferred to visit the initial training colour, indicating that the visual system of the bee does not judge colour simply on the basis the absolute number of photons captured by the three receptor classes. The results were reported to be consistent with the retinex theory of colour constancy, which holds that an algorithm using long-range interactions explain colour constancy (Land 1986; Werner et al. 1988), but did not exclude the possibility of colour constancy being explicable by a von Kries-type mechanism (Werner et al. 1988). This is because the stimuli colours used in a Mondrian-type experiment are not similar, and once some reasonable type of colour constancy mechanism is implemented it is not necessarily very difficult for a visual system to distinguish a target colour from the differently coloured distracters. A Mondrian-type experiment cannot therefore be interpreted as showing that an animal has perfect colour constancy because it is possible that the target colour has slightly changed, but it is still the most similar colour to that learned in the training conditions. Theoretical analyses of retinex-type constancy suggest that the generation of independent lightness values for each receptor is functionally similar to a von Kries type normalisation (Mausfeld 1998), and that this type of mechanism could also be limited by spectrally broad and overlapping photoreceptors (Worthey and Brill 1986). The results of this current study show that the problem of similar colours is very relevant to understanding mechanisms of colour constancy.

Flower shape as a cue to compensate
for inaccuracies of colour vision

When flowers are similar in colour, bees may use alternative cues to aid identification of which flowers they wish to visit. For example, Giurfa et al. (1994) showed that when target and distracter colours are similar, honeybees may use scent markings to help identify which flowers to visit.

We tested the addition of a shape cue to distracter flowers separated by a colour distance of 0.062 hexagon units. The bees used this visual cue to avoid distracters and chose target flowers with 100% accuracy, even when illumination conditions were changed. When shape was not available as a visual cue bees made a number of errors in choosing between similarly coloured flowers separated by a colour distance of 0.062 hexagon units, especially in spectrally variable illumination (Fig. 4B). This shows plants may evolve flowers of different shape to competing species to promote pollinator constancy. The shape cue avoids the problems that spectrally variable illumination places upon flower identification and may thus serve to be a more reliable cue in many natural foraging situations.

Conclusion

This study suggests that there is considerable pressure on plants to produce flowers whose colour signal is not similar to other flowers that bloom simultaneously, so

that the benefits of flower constancy can be maintained. The need for plants to produce distinctive flower colours makes it surprising that plants do not occupy all regions of bee-colour space with equal frequencies. Our results suggest that the inefficiency of colour constancy in the bees' colour visual system offers a likely explanation as to why bee blue flowers are poorly represented in nature compared with bee blue-green flowers. This shows that the psychophysics of bee colour vision can have important implications for plant evolutionary biology, and can help explain why natural flowers have the colours they do.

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