

RESEARCH ARTICLE

Avoidance of achromatic colours by bees provides a private niche for hummingbirds

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SUMMARY

That hummingbird-pollinated plants predominantly have red flowers has been known for decades, but well-investigated research studies are still rare. Preference tests have shown that hummingbirds do not have an innate preference for red colours. In addition, hummingbirds do not depend solely upon red flowers, because white-flowered hummingbird-pollinated plants are also common and temporarily abundant. Here we show that both white and red hummingbird-pollinated flowers differ from bee-pollinated flowers in their reflection properties for ultraviolet (UV) light. Hummingbird-pollinated red flowers are on average less UV reflective, and white hummingbird-pollinated flowers are more UV reflective than the same coloured bee-pollinated ones. In preference tests with artificial flowers, neotropical orchid bees prefer red UV-reflecting artificial flowers and white UV-nonreflecting flowers over red and white flowers with the opposite UV properties. By contrast, hummingbirds showed no preference for any colour in the same tests. Plotting floral colours and test stimuli into the honeybees' perceptual colour space suggests that the less attractive colours are achromatic for bees and therefore more difficult to detect against the background. This underlying colour preference in bees might provide hummingbirds with a private niche that is not attractive to bees.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/214/9/1607/DC1>

Key words: flower colour, hummingbirds, orchid bees, colour preference, sensory exclusion, private niche.

INTRODUCTION

Red flower colours has long been thought to determine the specific interrelationships between hummingbirds and the trochilophilous (hummingbird-pollinated) plants that they visit (Porsch, 1931; Grant, 1966; Raven, 1972; Bradshaw et al., 1995; Lunau and Maier, 1995; Cronk and Ojeda, 2008). Only a few flower-visiting insects, such as some butterflies and beetles, are attracted to red flowers (Dafni et al., 1990; Kinoshita et al., 1999). The absence of an expected innate preference for red colours in hummingbirds (Bené, 1941; Miller and Miller, 1971; Stiles, 1976; Goldsmith and Goldsmith, 1979; McDade, 1983; Delph and Lively, 1989) has fostered the idea that floral colours of bird-pollinated plants evolved mainly to discourage nectar-robbing insects, rather than to attract birds (Raven, 1972; Bradshaw et al., 1995; Lunau and Maier, 1995; Cronk and Ojeda, 2008).

Bees have trichromatic colour vision, based on three photoreceptor types maximally sensitive in the ultraviolet (UV), blue and green waveband. Flower-visiting birds, such as hummingbirds, have an advanced tetrachromatic colour vision with photoreceptor sensitivities peaking in the UV, blue, green and red waveband (Autrum and von Zwehl, 1964; Herrera et al., 2008), suggesting that bees are less able to detect red colours (Grant, 1966; Raven, 1972). Bees are very abundant visitors to flowers in the subtropical and tropical regions, and are the main competitors of hummingbirds for nectar (Castellanos et al., 2004; Rodríguez-Gironés and Santamaría, 2004; Freitas et al., 2006), but bees are often far less effective pollinators than hummingbirds (Roubik, 1982; Irwin and

Brody, 2000). Floral traits have a twofold role in pollinator attraction as well as in plant defence. Long-tongued bees are able to extract the nectar from the deep floral tubes of hummingbird-pollinated plants, but rarely pollinate the flowers (Castellanos et al., 2004; Rodríguez-Gironés and Santamaría, 2004). Some bees are even able to pierce the floral tubes and to take up nectar through the perforations without touching anthers and stigma. As a consequence, trochilophilous plants are pollinated more efficiently if their flowers attract hummingbirds, but at the same time exclude bees (Irwin, 2006). Resource partitioning in nectar sources between hummingbirds and bees is known to be mediated by differences in flower morphology (Raven, 1972; Castellanos et al., 2003; Rodríguez-Gironés and Santamaría, 2004). However, direct evidence for a role of sensory exclusion through floral colours is lacking. One prediction of the sensory exclusion through floral colour hypothesis is the occurrence of differences in the spectral reflection properties between trochilophilous and melittophilous (bee-pollinated) flowers. To test this prediction we recorded the spectral reflection in the ultraviolet and visual range of wavelengths of neotropical bee- and hummingbird-pollinated flowers. We considered red flowers as well as white flowers, as the latter is the second most frequent flower colour of hummingbird-pollinated plants (Porsch, 1924; Burr and Barthlott, 1993; Dziedziuch et al., 2003). Another prediction of the hypothesis is the difference in response to the colours by bees compared with hummingbirds. To test this prediction we performed colour preference tests with orchid bees and hummingbirds, using a set of test colours matching the

differences in the spectral reflection properties in trochilophilous and melittophilous flowers.

MATERIALS AND METHODS

Spectral reflection measurements

The spectral reflection of the test stimuli and of natural flowers was measured with a USB2000 spectrophotometer (Ocean Optics, Inc., Ostfildern, Germany) calibrated between 300 nm and 700 nm. Illumination was provided with a deuterium–halogen light source (D₂H; World Precision Instruments, Sarasota, FL, USA) emitting light between 215 nm and 1700 nm. Measurements were taken at an angle of 45 deg to the surface of the spot being measured (Chittka and Kevan, 2005). A pellet of barium sulphate was used as a white standard and a black film can was used as a black standard for recordings of the spectral reflection. Flowers were collected in botanical gardens of North Rhine Westphalia, Germany, and in the Atlantic rainforest of Sao Paulo, Brazil. The flowers were preserved in storage boxes with wet paper until measurement on the same day. Flowers were categorized as bee- or hummingbird-pollinated from literature information (see supplementary material Table S1). For this purpose we included only plant species in our analysis for which information about visitation and/or pollination by bees or hummingbirds was available. Plant species for which the literature stated only potential pollinators based on morphological conditions (pollination syndrome) were excluded. Also plant species for which the literature reported bees as well as birds as visitors or pollinators were excluded from this study (supplementary material Table S2). The sample size of these flowers is too small to include them in our study. In order to approximate the phylogenetic independence of the data the following procedure was applied: the means and standard errors of the spectral reflection curves were calculated for species belonging to the same genus and for genera belonging to the same family. Only the latter curves were used to calculate the mean spectral reflection curves. For statistical analysis we compared the spectral reflection of trochilophilous and melittophilous flowers in four distinct wavebands, i.e. UV (301–400 nm), blue (401–500 nm), green (501–600 nm) and red (601–700 nm), using an unpaired, two-tailed *t*-test (Table 1).

Choice experiments

Hummingbirds as well as orchid bees were offered the same set of artificial flowers in a multiple choice test. We used white UV-reflecting and red UV-reflecting colour stimuli [non-iridescent red feathers of the grey parrot, *Psittacus erithacus* (Linnaeus 1758) and white feathers of the mute swan, *Cygnus olor* (Gmelin 1789)] and combined both with either a UV-transmitting or a UV-absorbing transparent foil to assemble the four test colours (Fig. 1A,B).

Humans were unable to see any difference between the two colour stimuli in each of the pairs of red and white artificial flowers, which differed only in the UV spectrum. In the preference tests we presented eight artificial flowers (diameter 30 mm) to which Eppendorf tubes carrying a reward were appended in a manner that each of the four colour types was presented twice. The reward was 400 µl of the customary 7% nectar surrogate for hummingbirds (Avian Bird Food Products, Oosterend, Texel, Netherlands) and 10 µl of 50% honey water for orchid bees. The hummingbirds were not able to deplete the reward during the test intervals, whereas the reward in tests with euglossine bees was refilled after visits by bees. To prevent inaccuracies through position preferences the individuals were tested in eight trials with a pseudorandom placement of artificial flowers such that each of the eight artificial flowers was presented once in every position. For hummingbirds each trial lasted 10 min. For euglossine bees the trials lasted 90 min. Amounts of reward and duration of trials took into account the different foraging activity of the orchid bees compared with the hummingbirds. Seven individual hummingbirds [*Amazilia amazilia* (Lesson 1827) 1 male; *Eugenes fulgens* (Swainson 1827) 1 female, 1 male; *Thaumastura cora* (Lesson and Garnot 1827) 1 male; *Hylocharis cyanus* (Vieillot 1818) 2 female, 1 male] belonging to the subfamily Trochilinae were tested singly. The hummingbirds were bred in Germany and fed exclusively out of glassy colourless nectar tubes before and between the experiments. The hummingbirds were thus completely flower-naïve and not rewarded in association with colour cues before testing. Sixteen individuals of marked *Euglossa viridissima* (Friese 1899) (three females and 13 males) were tested simultaneously in a flight cage measuring 3 × 2 × 2 m. All choices of orchid bees of which the markers could not be ascertained after each feeding event were treated as choices of a single individual bee. The preference of the few bees caught in the wild did not differ from those bred in the flight cage, so the data from both were pooled. For statistical analysis of colour preferences a one-tailed Mann–Whitney *U*-test was performed according to our hypothesis that bees should prefer red UV-reflecting and white UV-absorbing artificial flowers as revealed from our spectral reflection measurements.

Perceptual bee colour space

To obtain a bee-subjective view of the natural and artificial flower colours we used the colour hexagon, a widely accepted model system for bee colour vision (Chittka, 1992) (Figs 1, 2).

For the calculation of colour loci in the colour hexagon a standard background of green leaves and a standard daylight illumination (D65) was used. The centre point of the colour hexagon represents the colour locus of the background to which the photoreceptor types are assumed

Table 1. Mean reflection of 63 neotropical red (r) and white (w) trochilophilous and melittophilous flowers in the ultraviolet, blue, green and red wavebands

Waveband	Flower colour	Mean reflection of melittophilous flowers	Mean reflection of trochilophilous flowers	<i>P</i>	<i>t</i>	d.f.
Ultraviolet	w	0.036	0.087	0.0067	2.957	25
	r	0.102	0.033	0.0001	5.164	19
Blue	w	0.258	0.220	0.3548	0.943	25
	r	0.118	0.032	0.0001	5.882	19
Green	w	0.329	0.242	0.0615	1.958	25
	r	0.195	0.041	0.0001	5.916	19
Red	w	0.336	0.277	0.2539	1.168	25
	r	0.488	0.255	0.0006	4.077	19

An unpaired two-tailed *t*-test was used for comparison of mean reflection values of trochilophilous and melittophilous flowers of the same human-visible floral colour. The mean spectral reflection was calculated for species belonging to the same genus and for genera belonging to the same family; only the latter reflection data were used to calculate the mean spectral reflection of each waveband.

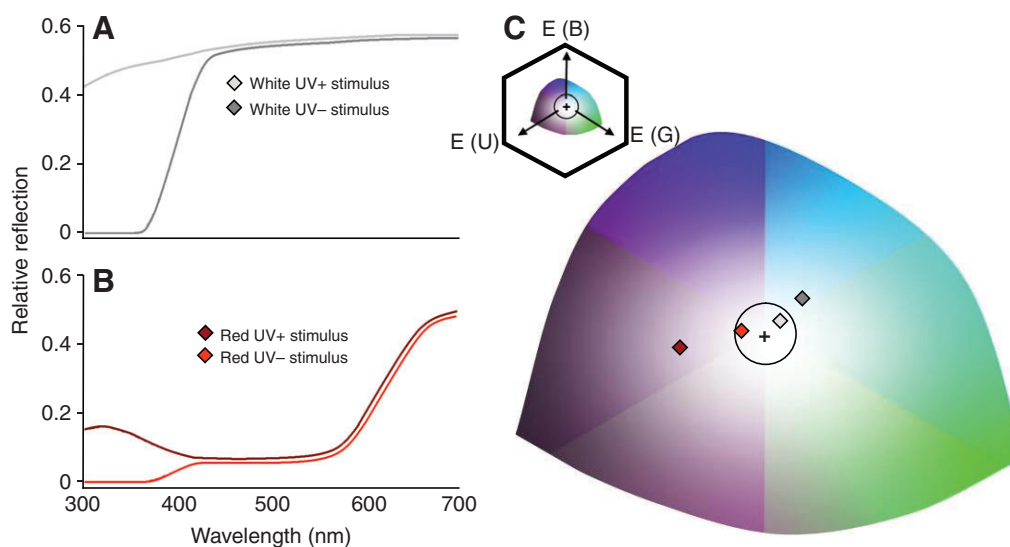


Fig. 1. Spectral reflection curves of the test stimuli and the representation of the respective colour loci in the honeybees' perceptual colour space. (A) Spectral reflection of white test stimuli. (B) Spectral reflection of red test stimuli. (C) Colour loci of red and white ultraviolet (UV)-reflecting and UV-non-reflecting test stimuli in the colour hexagon (Chittka, 1992). The centre point is indicated by +. The spectrum locus borders the coloured area within the colour hexagon. Achromatic colours with fewer than 0.1 hexagon units between the respective colour locus and the centre point are located in the circled area around the centre point. Inset: overview of the total colour hexagon. The excitations of the ultraviolet [E (U)], blue [E (B)] and green [E (G)] receptor types are indicated by arrows.

to be adapted (Chittka, 1992). The colour locus of a coloured object in the colour hexagon is generated by the calculated excitation of the UV, blue and green photoreceptors. The spectrum locus represents the colour loci of monochromatic colours and mixtures between the most extreme short- and long-wavelength ('bee-purple') colours. The spectrum locus borders the bee-visible colour space. Bees navigate and evaluate visual information of target objects by means of input exclusively to the green photoreceptor when an object is viewed at an angle of $<15^\circ$; if the visual angle exceeds 15° bees use input from all three photoreceptor types and exhibit colour vision (Spaethe et al., 2001). The green contrast was calculated as the excitation difference of the green photoreceptor produced by a given visual target stimulus and its background, i.e. the artificial flowers and the background used in the choice experiments or the natural flowers and a standard background of green leaves (Chittka et al., 1994), because green foliage is the prevalent background for most flowers. Most studies so far have shown that the absolute value rather than the direction of green contrast is relevant for orientation in bees (Giurfa et al., 1996; Spaethe et al., 2001). Searching time for flowers negatively correlates with the amount of green contrast between flower and background (Spaethe et al., 2001). Colour loci of achromatic colours are located close to the centre point, whereas colour loci of high colour purity are located close to the spectrum locus. Behavioural tests with honeybees and bumblebees – the standard systems for the study of colour perception and preference in bees – show that a minimum colour distance is needed to discriminate colour stimuli from the background (Chittka et al., 1994; Spaethe et al., 2001).

RESULTS

Reflection properties of red and white neotropical flowers

The mean spectral reflection curves show that white melittophilous flowers ($N=20$ species from 19 genera in 17 families) reflect less in the UV than trochilophilous flowers ($N=15$ species from 13 genera in 10 families) of the same colour group (Fig. 2A), and red melittophilous flowers ($N=5$ species from five genera in five families) reflect more in the UV than the respective trochilophilous

flowers ($N=32$ species from 25 genera in 16 families) that seem to be the same colour to the human eye (Fig. 2B). The individual species are listed in the supplementary information (supplementary material Table S1). In white flowers the differences in the spectral reflection between melittophilous and trochilophilous flowers are confined to the ultraviolet waveband, whereas in red flowers the spectral reflection of melittophilous flowers surpasses that of trochilophilous flowers in the UV, blue, green and red wavebands (Table 1).

Colour preferences of hummingbirds and neotropical bees

Hummingbirds did not show a preference for any of the four colour types of artificial flowers tested (white/UV absorbing vs white/UV reflecting; red/UV absorbing vs red UV reflecting; $P>0.05$; one-tailed Mann–Whitney U -test; Fig. 3A), whereas euglossine bees significantly preferred UV-reflecting red artificial flowers over UV-absorbing red flowers ($P=0.0036$, $U=66$; one-tailed Mann–Whitney U -test), and UV-absorbing white flowers over UV-reflecting white flowers ($P=0.0044$, $U=68$; one-tailed Mann–Whitney U -test; Fig. 3B). The data from the preference tests with euglossine bees and those with hummingbirds were pooled following a statistical test that showed that the individuals did not exhibit significant differences in their colour preferences.

Bee perceptual colour space

The colour loci of the artificial flowers less preferred by euglossine bees in the choice experiments were bee-achromatic as revealed in the perceptual colour space of the honeybee (Chittka, 1992) (Fig. 1C). The hexagon distance from the centre point of the UV-absorbing red test stimulus is 0.070 hexagon units and is smaller than that of the UV-reflecting red test stimulus, which was 0.279 hexagon units (Fig. 1C). The hexagon distance of the UV-reflecting white test stimulus was 0.078 hexagon units and is smaller than the UV-absorbing white test stimulus, which was 0.187 hexagon units (Fig. 1C).

The flowers of many trochilophilous plant families (31.3% of red, 40.0% of white) but none of the white-flowered melittophilous

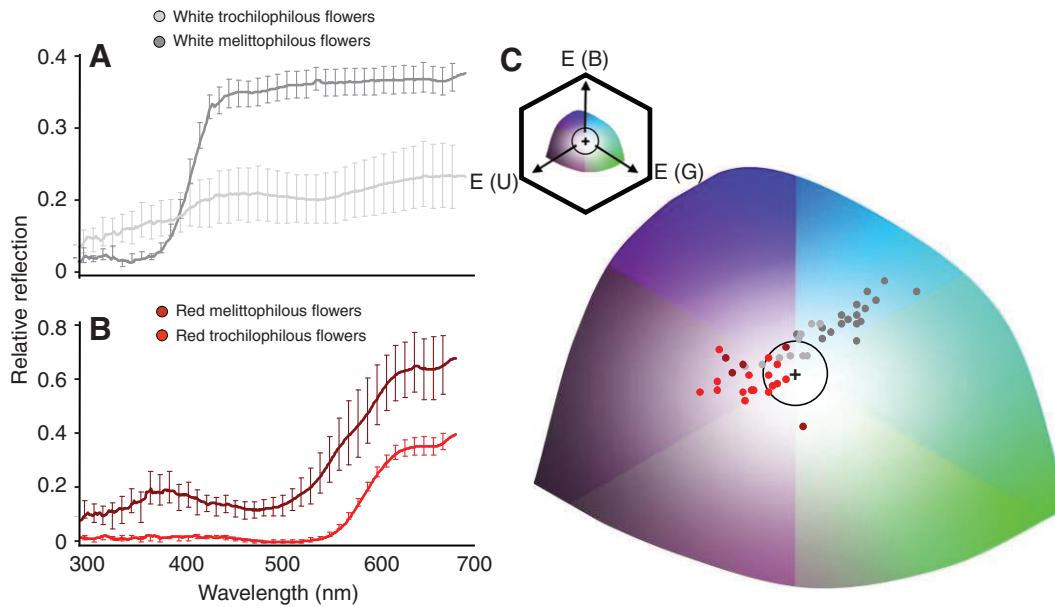


Fig. 2. Mean spectral reflection curves of hummingbird-pollinated and bee-pollinated neotropical flowers and the representation of the respective colour loci in the honeybees' perceptual colour space. (A) Mean spectral reflection of white trochilophilous and melittophilous flowers. (B) Mean spectral reflection of red trochilophilous and melittophilous flowers. The mean relative spectral reflection is given with the standard error. For calculation of the mean spectral reflection curves see Material and methods. (C) Colour loci of red and white melittophilous and trochilophilous flowers in the colour hexagon (Chittka, 1992). The centre point is indicated by +. Achromatic colours with fewer than 0.1 hexagon units between the colour locus and the centre point are located in the circled area around the centre point. Inset: overview of the total colour hexagon. The excitations of the ultraviolet [E (U)], blue [E (B)] and green [E (G)] receptors are indicated by arrows. Each plant family is represented by one colour locus.

plant families are achromatic for bees and only one of the red-flowered melittophilous plant species (Fig. 2C). The mean hexagon distance between the colour loci of white melittophilous flowers and the centre point was 0.199 ± 0.047 hexagon units and was greater than that of trochilophilous flowers of the same colour, which was 0.108 ± 0.032 hexagon units (Fig. 2C). The respective value of red melittophilous flowers was 0.135 ± 0.04 hexagon units and was greater than that of trochilophilous flowers of the same colour, which was 0.127 ± 0.054 hexagon units (Fig. 2C). For bees, the green contrast between the test colours and the background of the test stimuli was similar for the UV-reflecting and the UV-absorbing test stimuli (Table 2). The green contrast between white melittophilous flowers and the background of (standard) green leaves was greater than that of trochilophilous flowers, whereas the green contrast between red melittophilous flowers and the background of (standard) green leaves was less than that of trochilophilous flowers (Table 2).

DISCUSSION

It has been largely overlooked that red is not the exclusive floral colour of hummingbird-pollinated plants. Most non-red hummingbird-pollinated flowers, particularly among the Monocotyledonae, are white or pale pink (Porsch, 1924; Burr and Barthlott, 1993; Dzierżoch et al., 2003), or even undergo a colour change from green nectar-producing flowers to red non-rewarding flowers (Delph and Lively, 1989). In this study we demonstrate not only that neotropical red and white bee-pollinated and hummingbird-pollinated flowers differ in their spectral reflection properties, but also that orchid bees are less attracted to artificial flowers simulating the colour of trochilophilous flowers than to those of melittophilous flowers that appear identical in colour to human observers. Because hummingbirds do not exhibit a colour preference in tests with the same set of artificial flowers, the colour

preference of orchid bees may contribute to them not visiting trochilophilous flowers.

Reflection properties of red and white neotropical flowers

Previous studies on floral colour preferences in bees suggested that bees, despite their low sensitivity in the red range of wavelengths, visit red flowers even if they have no additional reflection in the ultraviolet waveband (Chittka and Waser, 1997). Our results do not conflict with these observations, but suggest that the attractiveness of red colours is enhanced by additional ultraviolet reflection. Forrest and Thomson showed that red floral colours must be interpreted together with the background against which they are presented (Forrest and Thomson, 2009). Bees take longer to detect UV-absorbing red coloured flowers if presented against a complex background such as natural green leaves (Forrest and Thomson, 2009).

Spectral reflection properties of flowers in temperate regions demonstrated that white melittophilous flowers regularly absorb UV light (Kevan et al., 1996), whereas red melittophilous flowers reflect UV light (Chittka and Waser, 1997). These findings are in accordance with our findings in neotropical flowers. The few UV-reflective white flowers might use alternative strategies to become conspicuous to bees, such as strong green contrast against the background or dissected shape (Chittka et al., 1994; Kevan et al., 1996). The spectral reflection of white trochilophilous flowers has not been systematically studied so far, but semi-quantitative UV photography revealed a high proportion of UV-reflective white trochilophilous flowers (Burr and Barthlott, 1993) and thus confirms our findings.

Colour preferences of hummingbirds and neotropical bees

Behavioural tests with hummingbirds have demonstrated the lack of innate colour preferences (Bené, 1941; Miller and Miller, 1971; Stiles, 1976; Goldsmith and Goldsmith, 1979; McDade, 1983; Delph and Lively, 1989). Bumblebees as well as honeybees exhibit innate

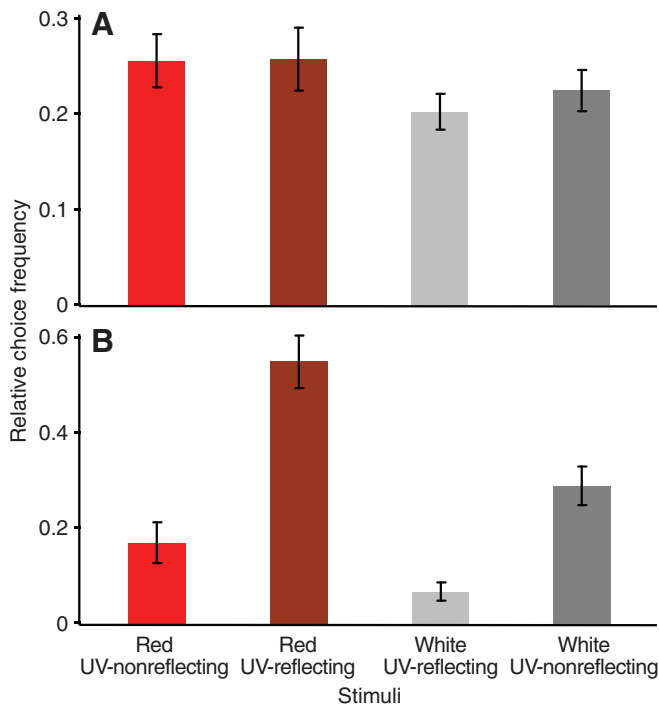


Fig. 3. Colour preferences of hummingbirds and euglossine bees for red and white artificial flowers with and without ultraviolet reflection. (A) Mean choice frequency of hummingbirds ($N=7$) for four types of simultaneously presented permanently rewarding artificial flowers, and (B) mean choice frequency of euglossine bees ($N=17$) for the same set of artificial flowers. The mean percentage of choice is given with the standard error.

colour preferences encompassing preferences for colours dominated by blue wavelengths (Giurfa et al., 1995; Gumbert, 2000), preferences for colours that contrast with the background (Giurfa et al., 1996; Lunau et al., 1996; Vorobyev and Brandt, 1997; Spaethe et al., 2001; Dyer and Chittka, 2004) as well as preferences for colours of high colour purity (Lunau et al., 1996). In addition, learning speed and learning capacity in bees are also dependent on colour (Menzel, 1967). Our colour-preference tests with neotropical orchid bees differed from those in the above-mentioned studies in that we used artificial flowers with only those colours that simulated the colour differences in the UV waveband of red and white trochilophilous and melittophilous flowers. Under these conditions, experienced orchid bees maintain preferences for distinct colours even in the absence of differentiating rewards. The maintenance of colour preferences in experiments with two different colour stimuli associated with the same amount of reward has previously been

demonstrated with bumblebees (Smithson and Macnair, 1996; Smithson and Macnair, 1997). The results of the comparative preference tests with orchid bees and hummingbirds closely correspond to those expected from the spectral reflection measurements. However, it is noteworthy that hummingbirds do not show reciprocal colour preferences to orchid bees; instead, they showed no colour preference at all, as in previous studies (Bené, 1941; Miller and Miller, 1971; Stiles, 1976; Goldsmith and Goldsmith, 1979; McDade, 1983; Delph and Lively, 1989). Given that hummingbirds do not exhibit any colour preferences, the colour preferences of orchid bees may contribute to them not visiting the trochilophilous flowers. From studies of honeybees and bumblebees it is known that workers take longer to detect achromatic colours, which prolongs their searching time and reduces foraging efficiency (Spaethe et al., 2001; Reisenman and Giurfa, 2008) and may result in a preference for easily detectable colours. In addition, the colour loci of many red, as well as white, hummingbird-pollinated flowers appear achromatic to bees and thus would provide only limited colour contrast against a background of green leaves (Chittka et al., 1994; Spaethe et al., 2001; Dyer and Chittka, 2004).

Bee perceptual colour space

The bees' perceptual colour space developed by Chittka has been successfully applied to quantify and visualize the perceptual colour contrast (Chittka, 1992). The colour hexagon is an equidistant colour space in which the distance between the colour loci of flower and background, as well as between two flowers, represents the perceptual colour contrast between the pairs of colour loci (Chittka, 1992; Chittka et al., 1994; Spaethe et al., 2001; Dyer and Chittka, 2004). Experimental studies have shown that bees can detect a coloured target against a background with a minimum perceptual distance exceeding 0.1 hexagon units; otherwise the colours appear achromatic to bees (Chittka et al., 1994; Spaethe et al., 2001). Our study shows that, using this criterion, many floral colours of red and white trochilophilous flowers appear achromatic to bees, whereas melittophilous flowers do not. Because the amount of green contrast between UV-reflecting and UV-absorbing target stimuli and background was similar in the choice tests, it was concluded that the observed preference of the orchid bees was independent of green contrast; this holds for red as well as for white test stimuli. Moreover, because white melittophilous flowers offer more green contrast to the background than white trochilophilous flowers, whereas red melittophilous flowers do not, green contrast does not seem to be an overall key parameter, which could explain the flowers' attractiveness to bees.

Hummingbird-pollinated plants benefit more from the exclusion of flower-visiting bees than *vice versa* (Castellanos et al., 2003), thereby favouring strategies of trochilophilous flowers to exclude

Table 2. Excitation values of ultraviolet (UV), blue and green photoreceptor types and values for green contrast for 63 neotropical red (r) and white (w) trochilophilous and melittophilous flowers and UV-reflecting and UV-absorbing artificial flowers used in the choice experiments

Flower type	Flower colour	Flowering syndrome/colour type	Photoreceptor excitation values			Green contrast values
			UV	Blue	Green	
Natural	r	Melittophilous	0.709	0.639	0.581	-0.081
		Trochilophilous	0.383	0.306	0.234	0.266
	w	Melittophilous	0.535	0.751	0.706	-0.206
		Trochilophilous	0.627	0.626	0.513	-0.013
Artificial	r	UV-reflecting	0.749	0.547	0.430	0.070
		UV-absorbing	0.370	0.449	0.426	0.074
	w	UV-reflecting	0.904	0.878	0.816	-0.316
		UV-absorbing	0.645	0.849	0.809	-0.309

bees. Besides colour, some other floral adaptations to visitation and pollination by hummingbirds are also known to exclude bees: absence of a landing platform, inappropriate size of the corolla tube, and low-concentration, dilute nectar (Raven, 1972; Irwin et al., 2004; Rodríguez-Gironés and Santamaría, 2004). In addition, hummingbird-pollinated flowers typically do not emit odours and thus lack chemical attractants typically associated with bee-pollinated flowers (Raguso, 2008). Our results show that the floral colours of hummingbird-pollinated plants are effective sensorial barriers that contribute to exclude bees from visiting these flowers. The colours of hummingbird-pollinated flowers thus have a dual function: the attraction of hummingbirds and the repellence of bees. Sensory exclusion of bees is not necessarily restricted to red and white colours, but may also be the case for pink, orange, green and blue flowers and for bird-pollinated flowers from the paleotropics.

Applying Possingham's model (Possingham, 1992) Rodríguez-Gironés and Santamaría predicted that resource-partitioning among bees and hummingbirds will develop solely based on the fact that bees require more time to find flowers of one species with a specific colour as compared with differently coloured flowers of another species (Rodríguez-Gironés and Santamaría, 2004). This prediction completely fits our results. In their study Rodríguez-Gironés and Santamaría assumed, that if bee visits were costly for the ancestral yellow and blue bird flowers, the yellow flowers would experience a selective pressure to become red (Rodríguez-Gironés and Santamaría, 2004). In this example they considered that, at any given time, this bird flower occurred in only two shades of colour, one of them with a slightly longer wavelength (an orange morph). By contrast our results indicate that the predicted main shift in the spectral reflection may have occurred in the UV waveband, and that pure red colours may be only one of several solutions to distract bees. Owing to the bees' preference for colours of high spectral purity (Lunau et al., 1996), the predicted colour shift is not restricted to longer wavelengths, but may also result in less spectrally pure colours (a pale morph or a dull morph). Moreover, hummingbirds learn to associate floral colours with expected rewards very well, whereas even experienced bees retain their innate colour preference to some degree (Smithson and Macnair, 1996; Pohl et al., 2008).

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