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ORIGINAL PAPER

Background complexity affects colour preference in bumblebees

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Abstract Flowers adapted for hummingbird pollination are typically red. This correlation is usually explained by the assertion that nectar- or pollen-stealing bees are "blind" to red flowers. However, laboratory studies have shown that bees are capable of locating artificial red flowers and often show no innate preference for blue over red. We hypothesised that these findings might be artefacts of the simplified laboratory environment. Using bumblebees (*Bombus impatiens*) that had been trained to visit red and blue artificial flowers, we tested whether colour preference was influenced by complexity of the background on which they were foraging. Many bees were indifferent to flower colour when tested using a uniform green background like those commonly used in laboratory studies, but all bees showed strong colour preferences (usually for blue) when flowers were presented against a photograph of real foliage. Overall, preference for blue flowers was significantly greater on the more realistic, complex background. These results support the notion that the red of "hummingbird syndrome" flowers can function to reduce bee visits despite the ability of bees to detect red and highlight the need to consider context when drawing inferences about pollinator preferences from laboratory data.

Keywords *Bombus* · Colour · Pollination syndromes · Ornithophily

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Introduction

Many hummingbird-pollinated plants exhibit a "syndrome" of flower characteristics that includes copious, dilute nectar; narrow, tubular corollas and, perhaps most obviously, red colour (Faegri and van der Pijl 1979; Thomson et al. 2000). Convergence on red in hummingbird-pollinated taxa (e.g. in *Penstemon*, *Lobelia*, *Castilleja*, *Ipomopsis* and *Aquilegia*; Grant 1994) is difficult to explain on the basis of bird preference because there is no evidence that birds have an innate proclivity for red (Grant and Grant 1968; Lunau and Maier 1995). Instead, its significance most likely lies in the relative insensitivity of bees to long-wavelength light (Proctor et al. 1996). In fact, bee insensitivity to red is such that researchers can work with bees under red light with little risk of the insects taking flight. Thus, the argument goes, the red of bird syndrome flowers serves less to attract bird visitors than to deter pollen-consuming or nectar-robbing bees that are inefficient pollinators of these plants (Porsch 1931, cited in Lunau and Maier 1995; Raven 1972; Thomson 2003).

However, bees are not blind to red (Chittka and Waser 1997). Virtually all studied species are trichromats with receptor sensitivities peaking around 340, 430 and 540 nm (ultraviolet, blue and green, respectively), although one species with an additional 600-nm receptor has been reported (Peitsch et al. 1992). However, the green receptor of most bees is stimulated (albeit weakly) by wavelengths up to 650 nm, well into the red. Distinguishing green from red should be difficult because both are detected by the same receptor, but bumblebees can be trained to select red disks on a green background (Chittka and Waser 1997). Furthermore, the evidence for an innate preference by bees for blue flowers over red has been mixed: Although several bumblebee (*Bombus*) species show the expected blue preference under laboratory conditions, some populations of the European *Bombus terrestris*, as well as North American *Bombus occidentalis*, are almost as likely to visit red flowers (Chittka et al. 2004; Raine et al. 2006). One study even noted a preference for red over blue artificial flowers by naïve North American *Bombus impatiens* (Gegear and Burns 2007). These observations challenge our understanding of how the red of hummingbird syndrome flowers could have evolved.

Rodríguez-Gironés and Santamaría (2004) suggested a resolution to this conundrum: Although red flowers may be easily detected by bees under laboratory conditions against uniform green backgrounds, they might be inconspicuous against the complex background provided by real foliage. We tested this hypothesis by training laboratory-reared bumblebees to visit red and blue artificial flowers and then allowing them to choose between "flowers" of these colours presented against (1) monochromatic green backgrounds and (2) photographs of real foliage.

Materials and methods

Colonies of captive-reared bumblebees (*B. impatiens* Cresson) were connected to a 2×2×2-m flight cage by a gated tunnel that allowed us to control entry and exit of individual bees. Lighting came from overhead fluorescent bulbs (flicker frequency, 120 Hz), including one 350-nm blacklight and two "natural-daylight" bulbs (colour temperature, 5,000 K); these were used to simulate more realistic, broader spectrum light than that provided by standard fluorescent lighting. During initial training, bees were allowed to forage freely from an equal number of red and blue artificial flowers until reliable foragers could be identified and individually marked. Pollen was provided to the hives daily. Training flowers were filled with 30% (*w*/*w*) sucrose solution (nectar) and were presented on a plain brown paper background. Flowers were constructed from clear 1.5-mL Eppendorf tubes with the lids removed, ringed by 3×3-cm square plastic "corollas" spray-painted either blue or red.

For testing colour preferences, we used arrays of ten blue and ten red equally rewarding flowers, each separated by 20 cm from its four nearest neighbours (see Electronic supplementary material, Suppl. 1) and embedded in 75×123-cm foam boards so that corollas were flush with the background. The boards were covered either by a matte, life-sized photograph of real foliage ("complex background"; see Electronic supplementary material, Suppl. 1) or by one of two different "simple" backgrounds: The first consisted of green construction paper of a type commonly used for experiments in our laboratory (uniform background); this was used for testing 12 bees from three different colonies ("A", "C" and "K"). However, because this paper differed slightly in texture and average chromaticity from the complex background, we could not be certain that treatment effects were due to background complexity per se. We therefore created a second simple background from the "complex background" photograph by serially applying the Gaussian blur tool in Adobe Photoshop® and shrinking and tessellating the resulting image; this "blurred background" was then printed on the same matte photographic paper used for the complex background and was used to test an additional five bees from a fourth colony ("J"). An equivalent number of bees from each of the four colonies was tested on the complex background. Reflectance spectra of flowers and backgrounds (Fig. 1a–c) were taken with an Ocean Optics USB 2000 spectrometer (Dunedin, FL, USA). A colour hexagon, showing locations in bee colour space of artificial flowers and points in the complex background (relative to the blurred background), was constructed following the methods described by Spaethe et al. (2001) (Fig. 1d).

Flowers were washed in warm water with detergent, rinsed and dried prior to a bee's first foraging bout to remove scent marks left by the previous bee. Bees were alternately assigned to background treatments. Immediately prior to testing, each bee was allowed to forage for at least one complete bout on an array of two red and two blue flowers, each containing 3 μL of nectar (refilled after being drained). These were arranged in a ~15×15-cm square on a brown background such that the two flowers of the same colour were diagonally opposite each other. This configuration ensured that the bee tended to visit red and blue flowers alternately and allowed us to verify that the bee was equally adept with both. For testing, each bee was observed foraging individually for four consecutive foraging bouts (approximately 100 visits in total). Flowers contained 3 μL of nectar at the start of the test and were replenished as soon as they were emptied by a bee. We recorded the number of visits to each flower colour, distinguishing rewarded from unrewarded visits (cases in which the bee landed on a flower but did not enter the tube far enough to obtain nectar).

We tested whether flower visits of individual bees deviated from a 50:50 ratio of red to blue using chi-square goodness-of-fit tests. It should be noted that this is a liberal test of preference because each flower visit is not completely independent [for instance, scent marks lingering from earlier visits by the bee might influence subsequent decisions—although this is more likely to affect decisions to enter a flower (rewarded visits) than decisions merely to land (unrewarded visits); Goulson et al. 1998]. To test for an overall effect of background on the proportion of visits to blue, we conducted Kruskal–Wallis tests using individual bees as replicates; we used a non-parametric statistic because data were not normally distributed. To evaluate changes in colour preference over time, we calculated Spearman rank correlations between the proportion of visits to blue flowers and bout number (out of four) for each bee; we then tested whether the

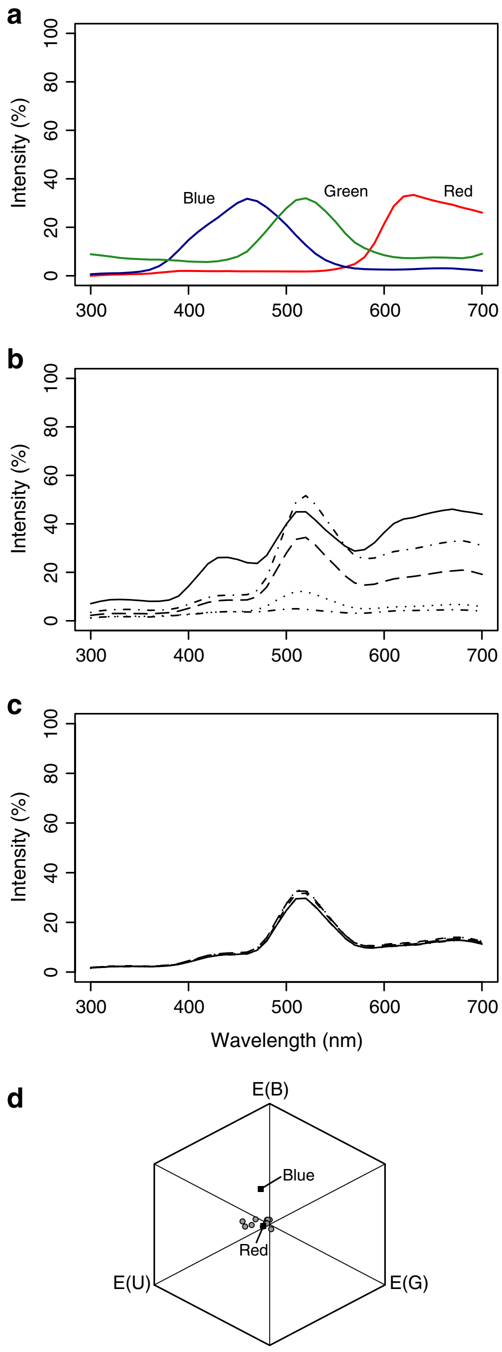

Fig. 1 Reflectance spectra and loci in colour space of materials used in experiment. **a** Red and blue artificial flowers and uniform green (paper) background; **b** five representative points in the complex (foliage photograph) background; **c** five points in the blurred background (homogenised foliage photograph). **d** Colour hexagon based on receptor sensitivities of *Bombus jonellus* (Peitsch et al. 1992) and a spectral function for "daylight" fluorescent bulbs (Wyszecki and Stiles 2000) with an additional 40-nm bandwidth peak at 350 nm. The origin represents the average chromaticity of the blurred background, *grey circles* represent 12 points in the complex background, and *black squares* represent the red and blue artificial flowers

set of correlation coefficients (ρ values) for all bees in a treatment differed significantly from zero using a Wilcoxon signed-rank test with individual bees as replicates. Analyses were done in R (R Development Core Team 2007).

Results

Most bees demonstrated a preference for one flower colour or the other, visiting significantly more of one colour than expected by chance. With monochrome backgrounds ("uniform" and "blurred"), six bees preferred blue, two preferred red, and nine were indifferent (Fig. 2a). On the complex background, blue preferences were stronger and nearly universal (one bee preferred red; Fig 2b). Thus, bees were more likely to show a significant blue preference when foraging on a complex background than when foraging on either the uniform background (colonies A, C and K: Fisher's exact test, odds ratio=0.074, P=0.027) or on the blurred photograph (colony J: Fisher's exact test, odds ratio=infinite, P=0.048). Overall, on the uniform background, bees made 51.5% of visits to blue flowers; on the complex background, 70.2% of visits were to blue (colonies A, C and K: Kruskal–Wallis χ^2=11.21, df=1, N_1=N_2=12, P=0.00081). This pattern also holds for bees from colony J: blurred background, 55.9% of visits to blue; complex background, 85.5% of visits to blue (Kruskal–Wallis χ^2=6.82, df=1, N_1=N_2=5, P=0.009). These results are qualitatively unchanged if unrewarded flower visits are excluded from analysis. Considering all bees, the proportion of visits to blue tended to decline over successive foraging bouts (mean Spearman's ρ=−0.31; Wilcoxon signed-rank test on correlation coefficients for all bees, V=32, N=34, P=0.036). The decline was slightly stronger in bees foraging on the simple backgrounds (going from an initial mean of 58.1% to 49.8% of visits to blue; mean ρ=−0.33, Wilcoxon V=32, N=17, P=0.036) and was not significant for bees on the complex background (79.8% to 72.6%; mean ρ=−0.29, Wilcoxon V=38.5, P=0.13).

Discussion

Backgrounds affect flower colour preferences. Uniform green backgrounds, intended to mimic natural foliage, are commonly used in laboratory studies of bee foraging (e.g. Forrest and Thomson 2008; Gegear and Laverty 2004; Smithson and Macnair 1996; Spaethe et al. 2001, but see Dyer et al. 2007) and may adequately represent some environments. However, depending on the question being asked, the use of such a simplified background could be misleading. For example, inferences about the likelihood of pollinator-mediated reproductive isolation between blue

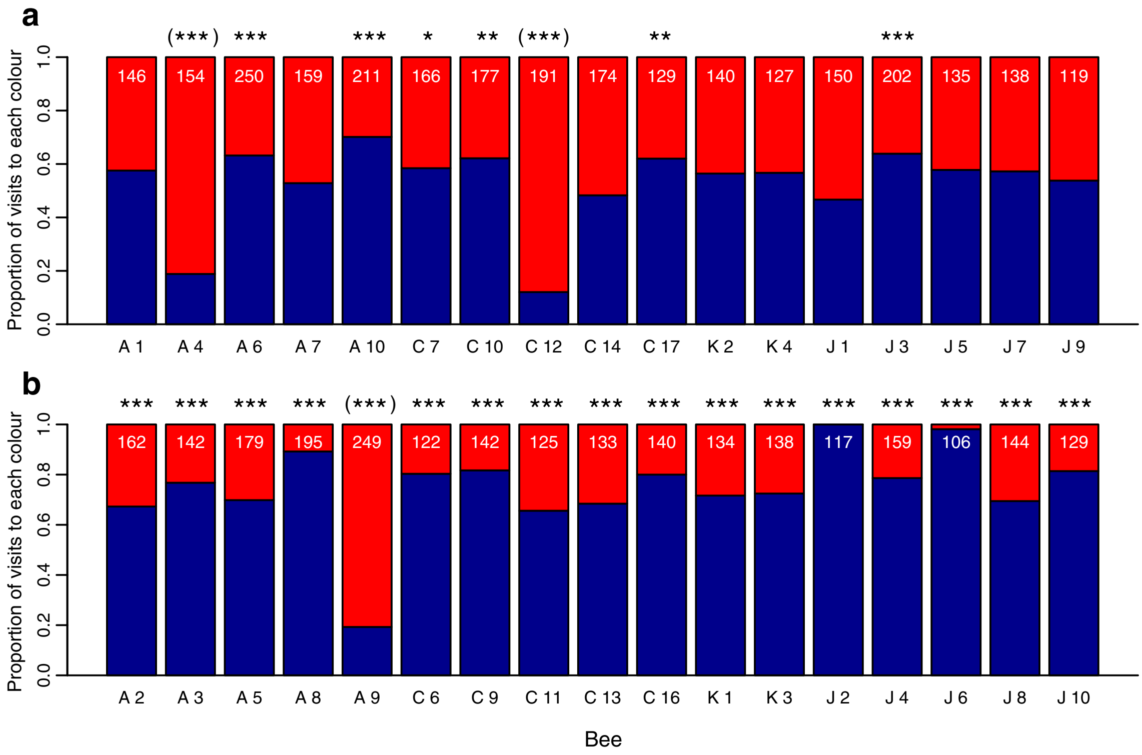

Fig. 2 Proportions of visits made by bees to flowers of each colour on simple (**a**) and complex (**b**) backgrounds. The *lower portion of each column* represents visits to blue; the *upper portion* represents visits to red. *Column labels* identify individual bees; *letters* represent different colonies. Only bees from colony J in the simple treatment foraged on the blurred background. *Asterisks over columns* indicate significant (*$P<0.05$, **$P<0.01$, ***$P<0.001$) preference for blue (or red, in *parentheses*). Number of flower visits made by each bee is given in *columns*

and red morphs depend on the strength of pollinator preferences (cf. Gegear and Burns 2007). Reproductive isolation would be impossible if pollinators foraged indiscriminately with respect to colour, as did many of the bees foraging on our simple backgrounds. Isolation is more likely if pollinators have strong colour preferences, as we saw on the complex background. Of course, most of our bees did not completely restrict visits to blue flowers even on the complex background. Additional factors reinforcing blue preference or constancy would presumably be necessary to eliminate gene flow between red and blue morphs.

Colour contrast between flowers and background is known to affect foraging speed in bumblebees (Spaethe et al. 2001). Given a choice, bees are expected to prefer flowers with strong background contrast because reduced search time will increase foraging efficiency. Our bees had no consistent preference for red or blue flowers when tested on a simple green background despite an earlier report of greater search time for red compared to blue flowers of similar size (28-mm diameter; Spaethe et al. 2001) and despite the weak colour contrast between our red flowers and green backgrounds (approximately 0.05 or 0.07 units in colour space between the flowers and the blurred or uniform background, respectively). Our result suggests that locating red flowers on such a background—or, at least, locating these relatively large red flowers (9 cm^2) at the high flower density we used (approximately 22 flowers per square metre)—presents no particular challenge for foraging bees. Chittka and Waser (1997) also showed that bees could easily be trained to visit red flowers on a simple green background. However, we did observe a decline in the proportion of visits to blue flowers as bees gained experience with the array, supporting the notion that blue preference results from blue flowers being more easily detected in an unfamiliar environment.

Nevertheless, the much stronger blue preference observed on the complex background suggests that red flowers are less apparent in that context. This effect is not obviously predicted from colour loci (Fig. 1d) and colour contrast with the background—which is, on average, identical between the blurred and complex backgrounds. Instead, we suggest that it occurs because the corolla outline can be less readily discriminated against the disruptive patterning of the foliage photograph. It is known that bees can associate patterns, as well as colours, with floral rewards (Srinivasan 1994), but pattern recognition may be more difficult when the background is also patterned. A similar phenomenon is well documented in red–green colour blind humans (dichromats) for whom the identification of red objects against green backgrounds is particularly challenging when lighting is uneven or contours are irregular (Mollon 1989).

As noted above, even on a complex background, individual bees made a substantial fraction (~30%) of visits to red flowers. In a more realistic, three-dimensional setting, bees might have had more difficulty locating red flowers than they did on our two-dimensional, regular array. Bees without any prior foraging experience might also have made different choices; we only tested bees that were accustomed to locating blue and red artificial flowers on the floor of the flight cage. On the other hand, bees often do forage on red flowers in nature, sometimes without the aid of odour cues (Chittka and Waser 1997). Real, three-dimensional flowers might provide a more distinctive outline than the artificial squares employed here. The importance of flower shape in the context of different backgrounds remains to be tested.

Our experiment supports the hypothesis that the red colour of "hummingbird syndrome" flowers is an adaptation for bee avoidance despite the ability of bees to perceive red flowers (cf. Bradshaw and Schemske 2003). As Rodríguez-Gironés and Santamaría (2004) pointed out, in the presence of hummingbird competitors, bees are likely to visit blue flowers in preference to red because of their greater efficiency in harvesting nectar from blue flowers. Blue preference in our bees presumably resulted simply from the relative inconspicuousness of red flowers on a complex background. In an environment with competitors that have no such limitations in detecting red, nectar depletion in red flowers would tend to reinforce blue preferences in bees. In our experiment, of course, bees never encountered drained flowers.

Hummingbird preferences might also depend on context; naïve birds that are indifferent to red in the laboratory may find it more conspicuous than other colours when foraging under natural conditions (Chittka and Waser 1997). If hummingbirds indeed show greater red preference in more complex environments and bees show greater blue preference in the same conditions—as we have shown—the commonness of red in "hummingbird flowers" becomes easier to understand.

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Research was conducted in compliance with Canadian laws.

Conflict of interest The authors declare that they have no conflicts of interest.

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