

The Ultraviolet Colour Component Enhances the Attractiveness of Red Flowers of a Bee-pollinated Plant

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Keywords: Bee pollination; colour vision; floral colour evolution; pollination syndrome; red flower; UV reflection.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1093/jpe/rtaa023

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ABSTRACT

Aims Bee-pollinated flowers are rarely red, presumably because bees (which lack red receptors) have difficulty detecting red targets. Although the response of bees to red colour has been investigated in lab experiments, most stimuli have been pure red, while the subtle diversity of red as perceived by humans (human-red) has received very limited attention. Here we test the hypothesis that UV reflected from human-red flowers enhances their attractiveness to bees, through increased chromatic contrast.

Methods Using *Onosma confertum* (Boraginaceae), a plant with UV-reflecting red flowers that are pollinated by bumblebees, we investigated the effects of UV reflection on pollinator responses by conducting phenotypic manipulation experiments in the field. Colour preferences of flower-naïve bumblebees were also examined. Colour perception by bumblebees was estimated in terms of chromatic and achromatic contrast, based on two different colour perception models.

Important Findings We found that both natural and flower-naïve bumblebees strongly preferred visiting UV-reflecting targets compared with UV-absorbing ones. Colour models show that the UV-reflecting flowers exhibit higher spectral purity and higher chromatic contrast against the foliage background, whereas they have similar achromatic contrast in terms of green receptor contrast. These results indicate that the component of UV reflection increases chromatic contrast in *O. confertum*, enhancing the visual attractiveness of these red flowers to bumblebees. We further infer that the secondary reflectance might be a necessary component in human-red flowers that are primarily pollinated by animals without red receptors, such as bees.

INTRODUCTION

Colour is an important floral trait that mediates plant-pollinator interactions (Bradshaw and Schemske, 2003; Fenster *et al.*, 2004). For example, red-coloured flowers are often pollinated by birds (reviewed in Cronk and Ojeda, 2008). The red colour has been considered to serve two functions, i.e., to attract birds and to exclude bees (Raven, 1972). Bees are supposed to be excluded because they are often ‘undesired’ visitors to bird-pollinated flowers due to their relatively low efficiency as pollinators (Castellanos *et al.*, 2003; Thomson and Wilson, 2008; Krauss *et al.*, 2017; Lehmann *et al.*, 2019) or because they act as pollen thieves (Irwin *et al.*, 2010; Rojas-Nossa *et al.*, 2016). Red flowers might exclude bees because they lack receptors sensitive to red light (Peitsch *et al.*, 1992). This prediction has been supported by a number of studies (Lunau *et al.*, 2011; Bergamo *et al.*, 2016; Gegeer *et al.* 2017). However, it is common that primarily bird-pollinated flowers are also visited by bees (ref in Chittka and Waser, 1997; Martínez-Harms *et al.*, 2010), implying the signal exclusion is not perfect. Furthermore, not all red flowers are pollinated by birds, whereas they have rarely been studied in other pollination systems. Despite their rarity, bee-pollinated red flowers do indeed exist (Chittka *et al.*, 1994). Studies on these non-bird pollinated red flowers may provide new insights into our understanding of the evolution of flower colour.

Humans’ colour vision differs from bird or insect pollinators by lacking receptors that are sensitive to UV light (Briscoe and Chittka, 2001). The red floral colour as perceived by humans (human-red) includes two general types of reflectance spectra, depending on whether there is a secondary reflectance in the short wavelength (e.g., UV) region in addition to the primary red reflection (Chittka and Waser, 1997). The UV-absorbing and UV-reflecting red colours appear the same to human eyes, but are clearly different for many birds and bees, which are sensitive to UV light (Briscoe and Chittka, 2001). The responses of bees to red targets have been investigated in a number of lab experiments, but most of these targets have been pure red, without secondary

reflectance (Gegear and Burns, 2007; Martínez-Harms *et al.*, 2010; Telles *et al.*, 2017). Empirical studies that consider the diversity of human-red colours are very limited (see Lunau *et al.*, 2011).

Bees have trichromatic colour vision based on three photoreceptor classes maximally sensitive in UV, blue and green regions of the spectrum (Peitsch *et al.*, 1992). Pure red flowers will moderately stimulate the green receptor near the periphery of its spectral sensitivity. Together with a moderate stimulation of UV and blue receptors, this results in roughly equal signals in all receptors, forming an achromatic perception (Chittka and Waser, 1997). Such flowers may be detected by bees via an alternative (achromatic) mechanism through the contrast induced on the green receptor (Giurfa *et al.*, 1997; De Ibarra *et al.*, 2000; Reisenman and Giurfa, 2008), but this achromatic contrast is still low (Lunau *et al.*, 2011). These pure red flowers are usually hummingbird-pollinated, and are only visited secondarily by bees (references in Chittka and Waser, 1997). However, red flowers with secondary reflectance peaks in the UV and/or blue region are perceived by bees as having distinct colour, as all receptors except the UV and/or blue receptor are only weakly excited (Chittka *et al.*, 1994).

UV signals can make a target either more or less attractive to bees, depending on whether it increases the colour contrast (Chittka *et al.*, 1994; Kevan *et al.*, 2001) or the spectral purity (Lunau and Maier, 1995; Rohde *et al.*, 2013). For example, the UV colour component in yellow flowers has been suggested as being attractive to insect visitors by forming a high contrast pattern (Koski and Ashman 2014; Papiorek *et al.*, 2016). However, compared with UV-absorbing white flowers, UV-reflecting white flowers display a colour of low spectral purity for bees, decreasing their attractiveness (Lunau *et al.*, 2011). Theoretically, the presence of UV reflection in red flowers should increase their chromatic contrast against the background (Chittka *et al.*, 1994), so enhancing flowers' attractiveness to bees. However, the effects of the UV component of red flowers on pollinator behaviour have rarely been examined (Kugler, 1947).

Onosma confertum W. W. Smith is a bumblebee pollinated perennial herb distributed in SW China (Fig. 1a). It produces human-red flowers with a secondary reflectance peak in the UV region, which extends into the blue range. Using this plant species as a model, we studied the effect of the UV component in red colour on pollinator responses by conducting a phenotypic manipulation experiment under natural conditions. As a premise, we first confirmed that the manipulation effectively modified the floral colour as we expected. We then estimated how the red colours with and without the UV component would be perceived by bees in both chromatic and achromatic channels, using two widely used colour models. We further investigated whether the pollinators, bumble bees, showed any preference for the UV-reflecting red flower under natural conditions. To control pollinators' experience, we further used commercial bumblebees to test their naïve preference of different red colours (pure-red vs. UV+ red) using red petals from cultivated *Camellias*. Our results are discussed in the context of colour perception and the evolution of floral colour.

MATERIAL AND METHODS

Material and the study site

Onosma confertum (Boraginaceae) is a perennial herb with pendant tubular red flowers. The flowers produce copious nectar and are pollinated mainly by bumblebees (e.g., *Bombus friseanus*). Field experiments on *O. confertum* were carried out in the Shangri-La Alpine Botanical Garden (SABG), located in NW Yunnan province, SW China (27°54'N, 99°38'E, 3355m a.s.l.). The garden is aimed at protecting the local original vegetation from grazing, where *O. confertum* grows. To our knowledge, no nectarivorous birds occur in the area.

Two varieties of *Camellia japonica* were used in the experience-controlled bumblebee behavioural experiment. These plants have similar red flowers to human, but differ from each other in their

reflectance at the UV region. This experiment was conducted in Kunming Botanical Garden (KBG, 25°08'N, 44°25'E, 1960m a.s.l.), where many varieties of *C. japonica* are available.

UV reflection manipulation and colour measurement

We modified the methods described by Andersson and Amundsen (1997) and Johnson and Andersson (2002) to manipulate the reflectance of the UV region of *O. confertum* flowers. Parsol MCX and Parsol 1789 sunscreens were mixed equally and dissolved in aloe oil, then heated gently. This sunscreen mixture was gently spread onto the outer surface of all the flowers of each inflorescence, forming UV-absorbing red (hereafter UV-). This manipulation diminished the UV reflection (300 nm – 400 nm) effectively, leaving the shape of the human-visible part of the spectrum almost unchanged (Johnson and Andersson, 2002). To deal with the potential change in the floral texture, the substrate (i.e., the aloe oil) was also applied to the flowers of control inflorescences (UV-reflecting red, hereafter UV+), forming the same texture as the UV- flowers without any obvious change in the spectral shape (see Johnson and Andersson, 2002 and Koski and Ashman, 2014). To control the potential scent (although not detected by humans) of the sunscreen mixture, we spread the mixture onto the pedicels of each flower in the UV+ inflorescence treatment.

The reflectance spectrum of these treated flowers were measured using a spectrometer (USB Ocean Optics 2000+) equipped with a DH-2000 light source (both by Ocean Optics Inc., Dunedin, FL). Fifteen flowers (each from a separate individual) were collected and measured within one hour. Specifically, we first measured the exterior side of corolla of the intact natural flowers as a reference. Then the flowers were manipulated as UV+ and UV- successively, using the method described above, and the reflectance spectra were measured accordingly. We thus acquired three spectra data sets (natural, UV+ and UV-) for each flower and a sample size of 15 for each group. For each sample, we measured three times and then obtained the average. In addition, 20 green leaves from *O.*

confertum and several accompanied species were measured and averaged to represent the background. During measurement, the probe was held at 45° to the target surface. The probe and the object was controlled at a constant distance of about 2 mm.

Pollinator preference in the field

To estimate the effects of UV manipulation on floral attractiveness, the first flower choice of pollinators was observed in the field. A pair of inflorescences with similar numbers of open flowers were manipulated as UV+ and UV-, and then cut and placed in freshwater (in a small plastic tube) to keep them fresh. These inflorescences were sited 0.3 m apart from each other in grassland with a low density of *O. confertum* in order to reduce the effects of any previous experience of the pollinator. The inflorescences were replaced every two hours to keep them fresh and rewarding, and to maintain the effectiveness of the sunscreen mixture. This also reduced the potential effect of scent marks on flowers deposited by bumblebees, which may repel subsequent visitors (Gawleta *et al.*, 2005). To avoid the situation of one visiting individual being sampled many times, observations were carried out in three different locations that were far apart (ca. 1.0 km) from each other. The first flower choice (UV+ or UV-) of the visitors was observed, and whether they approached or actually landed on the inflorescences was recorded. Observations were carried out between 1 August and 7 August, 2015, during the full-blossom period of the species, from 10:30 to 16:30 (42 hours in total).

Colour choice by flower-naïve bumblebees

Although we tried to reduce the effect of pollinators' previous experience on their response in the field experiment (mentioned above), this effect was difficult to exclude under natural conditions. Therefore, three colonies of flower-naïve commercial bumblebees (*Bombus terrestris*, Biobest Ltd.) were used to examine their innate colour preference. These bees were trained and tested in Kunming

Botanical Garden (KBG). The choice of bumblebees was examined by offering four targets simultaneously. These four targets were arranged in a rectangular pattern, 10 cm apart, fixed on a background of complex natural green leaves (30 cm x 30 cm, printed from photos captured in the SABG, as suggested by Forrest and Thomson, 2009). The training and testing were conducted in a net flight cage (2 m x 2 m x 1.5 m) under natural light.

In the training stage, green paper discs of 3 cm diameter were used as targets (Fig. 2). In the centre of each was fixed a 200 μ L Eppendorf tube containing 200 μ L 50% sugar water that provided a permanent reward. Marked bees were released from a plastic tube connected to their hives at a distance of ca. 50 cm from the target plates and were enticed to one of the targets at random. This distance simulates the natural conditions over which bees use their achromatic (far distance, larger angle) and chromatic (close distance, smaller angle) vision mechanisms on approaching a flower (Guirfa *et al.*, 1997). Some bees then learned to forage on the targets after several training bouts. They flew back spontaneously after consuming the sugar water or were taken back to the hive using a plastic tube.

In the testing stage, green paper discs were replaced by UV+ and UV- red *Camellia* petals with the same shape and size as the training stage (two of each type, randomly intermixed and arranged). Two varieties of *C. japonica* collected from KBG were used in this experiment for two reasons. First, it is difficult to transport flower-naïve *B. terrestris* to SABG (where *O. confertum* grows) and *vice versa*. Second, these *Camellia* petals appear uniform to humans but differ in their UV reflectance (either UV+ or UV-, (Fig. 2), providing ideal material that may have more general implications. The *Camellia* flowers we used have no detectable scent, but we did notice that some varieties of *C. japonica* are fragrant (although most of them are not coloured red, e.g., the white-flowered *C. japonica* cv. 'Scented Sun', Jullien *et al.*, 2008). To reduce any potential difference due to a human-undetectable scent, a petal of a different group was placed under the testing target (e.g., for UV+ target, a UV- petal in same shape and size was covered by a UV+ petal). Targets and their

positions were changed after each foraging bout, both to remove scent marks left by the previous bee, and to avoid any potential position preference. All bees were tested individually to prevent competition by controlling the tube connected to the hive. The choice was counted when a bee approached (< 5 cm) or landed on one of the four targets. The first three bouts of each individual were used and only the first choice of each bout was counted.

Statistical analysis

Colour perception models We used both the colour hexagon (CH) model (Chittka, 1992) and the logarithmic version of the receptor noise-limited (RNL) model (Vorobyev and Osorio, 1998) to estimate the colour perception of bumblebees. Given that the receptors' spectral sensitivity is conservative in bees (Chittka, 1996), we used the data for *B. terrestris* from Peitsch *et al.* (1992). The receptor noise values of this bee species were obtained from Skorupski and Chittka (2010) as follows: $e_{UV} = 0.74$, $e_B = 0.61$ and $e_G = 0.67$. The raw floral reflectance spectra data were processed to 5 nm intervals between 300 nm and 700 nm. Daylight D65 (ICE) was used as the irradiance spectrum. These data were used to map the floral colours (reflectance spectra) into the colour models of bumblebees. The detailed calculation of colour loci coordinates can be found in the appendix of Renoult *et al.* (2017) for both the CH (formula A1.2 + A1.4 + A1.7 + A1.19) and RNL (formula A1.2 + A1.4 + A1.6 + A1.24) models. Mean reflectance of leaves from *O. confertum* and accompanied species were used as the background.

Chromatic contrast For both the CH and RNL models, chromatic contrasts are indicated by the Euclidean distances between pairs of colour loci in particular colour spaces, with greater distances indicating higher contrasts. In the CH model, this distance is given in hexagon units (HU) (Chittka, 1992), whereas in RNL model, in just noticeable difference units (JND) (Vorobyev and Osorio, 1998). We first calculated the pairwise colour distances among the UV+, UV- and natural flowers to

estimate the difference between these colours as perceived by bumblebees. We also calculated the distances from colour loci to the origin of the coordinates (namely the background) to estimate the colour conspicuousness of flowers. These values were compared among groups using one-way ANOVA followed by a Tukey test.

Spectral purity It has been suggested that some bee species prefer colours with higher spectral purity under some conditions (Rohde *et al.*, 2013). We estimated spectral purity in the CH model, dividing the distance between floral and background loci by the distance between corresponding monochromatic light and background loci (Lunau *et al.*, 1996). These values were compared among groups using one-way ANOVA followed by a Tukey test.

Achromatic contrast We used the contrast in the green receptor of bees to estimate achromatic contrast, calculated by the quantum catch of the green receptor produced by a stimulus, divided by that produced by the background (Giurfa *et al.*, 1997). A value of 1 indicates that the stimulus appears equal to the background for the receptor concerned; values < 1 or > 1 indicate that the stimulus appears dimmer or brighter than the background, respectively. These values were compared among groups using one-way ANOVA followed by a Tukey test.

Behavioural experiments For both the observation of experienced pollinators in natural conditions and the experimental test using naïve bumblebees, the responses (approaching or landing on the flowers) of bees were compared by Chi-square tests, to examine whether one type of target (*O. confertum* flower or *C. japonica* petal) was chosen by bumblebees with a significantly higher frequency than random (50%).

RESULTS

Floral colours

Properties of reflectance spectra - The reflectance spectra of natural flowers are shown in Fig. 1a. There is a strong reflection in the red region (600 nm - 700 nm) and an obvious secondary peak mainly in the UV region (300 nm - 400 nm), which extends to the blue region (400 nm - 500 nm). The shape of the floral reflectance spectrum in the UV+ group (with only the oil substrate applied) was unchanged, although the general brightness (total reflectance) was reduced. In contrast, for the UV- flowers, the sunscreen mixture reduced the reflectance in the UV region significantly, leaving other parts of the spectrum almost the same as the UV+ flowers. Although this colour was not pure red due to the presence of blue reflection, the chromatic contrast was nevertheless greatly reduced (see below), allowing us to estimate the function of the UV reflection component in floral attractiveness. The flowers from the two groups compared, i.e., UV+ and UV-, exhibited similar brightness in most parts of the spectrum, except the UV region.

The floral colour loci mapped in the CH and RNL colour space are shown in Figs. 1b and 1c, respectively. In the CH model, a large number of the UV- floral loci fell within the 0.1 diameter circle in the coordinate centre, indicating it to be difficult for bees to distinguish them as chromatic signals. In contrast, the UV+ and natural flowers had loci in the region of the UV and UV-blue boundary farther from the centre, indicating that these flowers could be recognised as chromatic targets. The RNL model showed a similar pattern, on which UV- floral loci were closer to background locus in comparison with natural and UV+ flowers.

Chromatic contrast - The chromatic aspect of UV+ and natural floral colours were very similar as perceived by foraging bumblebees. The differences in colour distance between UV+ and natural flowers are rather low, with 0.026 ± 0.003 HUs (mean \pm s.e.) in the CH model and 0.095 ± 0.016 JNDs in the RNL model, respectively. Thus, these two groups of colours should appear similar to bumblebees. However, bumblebees are readily able to distinguish between the UV+ and UV- flowers. Both models showed the distance between these two colours to be much higher (0.156 ± 0.016 HUs in the CH model and 0.806 ± 0.074 JNDs in the RNL model).

For both the CH and RNL models, there were significant differences in the mean distance from the colour loci to the background among three groups (one-way ANOVA, $F_{2, 42} = 23.336$, $P < 0.001$ for the CH model and $F_{2, 42} = 65.780$, $P < 0.001$ for the RNL model). The multiple comparisons showed similar patterns in both colour models, with significantly higher contrast in UV+ than in UV- flowers (0.144 ± 0.005 vs. 0.092 ± 0.005 in CH model, 0.761 ± 0.020 vs. 0.479 ± 0.024 in RNL model) and no significant difference between UV+ and natural flowers (0.144 ± 0.005 vs. 0.128 ± 0.006 in CH model, 0.761 ± 0.020 vs. 0.821 ± 0.022 in RNL model), (Fig. 3a).

Spectral purity – As perceived by bumblebees, the spectral purity differed significantly among the three groups (One-way ANOVA, $F_{2, 42} = 78.575$, $P < 0.001$). The mean value of UV+ flowers was significantly higher than UV- flowers (0.400 ± 0.014 vs. 0.116 ± 0.017), whereas, the difference between UV+ and natural flowers was not significant (0.400 ± 0.014 vs. 0.362 ± 0.015), (Fig. 3b).

Achromatic contrast – Pairwise difference in green receptor contrast was 0.825 ± 0.125 between untreated and UV+ flowers, and 0.239 ± 0.056 between UV+ and UV- flowers. Green receptor contrasts among flowers differed significantly among the three groups (one-way ANOVA, $F_{2, 42} = 21.115$, $P < 0.001$). The mean value of green receptor contrast stimulated by natural flowers (2.188 ± 0.146) was significantly higher than the UV+ and UV- flowers, as the manipulation inevitably decreased the total brightness of floral colour (Johnson and Andersson, 2002), whilst there was no significant difference between the latter two groups (1.362 ± 0.144 vs. 1.124 ± 0.099), (Fig. 3c).

Behavioural experiments

Pollinator preference in the field - *Bombus friseanus* was the only visitor recorded during observations. We recorded 84 approaches in total, regardless of whether or not bees eventually landed on the flowers. Bumblebees strongly preferred approaching the UV+ inflorescences with 59 out of 84 doing so (Fig. 4a), ($\chi^2 = 27.52$, $P < 0.001$). Those bumblebees that actually landed on the

flowers ($N = 61$) exhibited a similar preference pattern with 50 out of 61 doing so (Fig. 4a), ($\chi^2 = 14.66$, $P < 0.001$).

Preference of flower-naïve bees - Sixteen flower-naïve bees were involved in experiments (eight, four and four from each colony, separately). They exhibited a significant preference for UV+ targets over UV- ones. Specifically, UV+ received 42 out of the 57 visits (Fig. 4b), which is significantly higher than expected if visits were random ($\chi^2 = 6.136$, $P = 0.013$).

DISCUSSION

UV reflection and floral attractiveness of *Onosma confertum*

Most Hymenoptera do not possess red receptors (Peitsch *et al.*, 1992), and therefore cannot perceive pure red flowers using the chromatic channel in most circumstances (Lunau *et al.*, 2011). Although bees can perceive these flowers using achromatic contrast induced on the green receptor (Chittka & Waser, 1997), these pure red colours are less attractive due to reduced detectability. Our results show that, bumblebees were strongly attracted to UV-reflecting flowers in comparison to those UV-absorbing ones. This preference was found both in the field and under experience-controlled conditions, implying that UV-reflecting red was favoured against pure red by both experienced and naïve bumblebees. Actually, the secondary reflectance of *O. confertum* flowers extends to the blue region, which became more evident in UV-absorbing manipulated flowers, where it increased its chromatic contrast to some extent compared to pure red colour. Even under this situation, a lack of UV reflection still reduced the attractiveness of the flowers. Notably, although the flower-naïve bumblebees had been trained to visit achromatic stimuli (green) that are more similar to the UV- test target (Fig. 2), they preferred the UV+ target in the testing stage.

An achromatic mechanism may not be responsible for this response difference, as there was no significant difference between the green receptor contrast with both UV-reflecting and UV-absorbing targets in our experiments. This is possibly because the green receptor has relatively low sensitivity in the UV region. Furthermore, it has been suggested that the achromatic mechanism is not used as a major cue for stimulus detection by diurnal insects (Spaethe et al., 2001; van der Kooi et al., 2019). Instead, the different responses of bumblebees may be due to differences in the chromatic mechanism (Dyer & Chittka, 2004). Both colour models indicated that the UV reflection in human-red colour increased the chromatic contrast of flowers against the leaf background. The spectral purity was higher in UV-reflecting red flowers as well, which has been suggested as a more attractive signal to some bees (Rohde et al., 2013). However, at least in the present study, the effects of spectral purity cannot be disentangled from colour contrast (van der Kooi et al., 2019). The UV reflection is no more important than reflectance of other parts of the spectrum (Kevan et al., 2001), but some pollinators have been reported to show an innate preference to such a signal (Dyer et al., 2019). In the case of red flowers, the UV reflection may add a chromatic cue for bees during visual detection.

The evolution of red flowers

Plant species with red flowers are common in nature, but very few of them are pollinated by bees. Besides *O. confertum*, we noticed that the red poppy (*Papaver rhoeas*) is also bee-pollinated in Europe (McNaughton & Harper 1960; Proctor et al., 1997). Given that bees lack red receptors, it is reasonable to predict that the secondary reflectance may be a necessary component of these red flowers to increase the chromatic contrast (see also Lunau et al., 2011). In accordance with this prediction, flowers of both *Onosma confertum* (this study) and *Papaver rhoeas* (in Europe) are UV-reflecting (Chittka & Waser, 1997; van der Kooi & Stavenga 2019). In a preliminary cross-

continental survey, secondary reflectance was found in all the ten bee-pollinated red flowers we collected (Zhe Chen and Yang Niu, unpubl. data). However, we noticed that two out of four red flowers that are pollinated by bees have been reported to be UV-absorbing from a natural community in Brazil (Camargo et al., 2019). For those pollinated by animals that are equipped with a red receptor, the pattern is more difficult to predict. For example, beetles in family Glaphyridae are equipped with R receptors (Martínez-Harms *et al.*, 2012). A number of Mediterranean red flowers that are pollinated by these beetles lack the secondary reflectance (Dafni *et al.*, 1990). Interestingly, these beetle-pollinated flowers also include *Papaver rhoeas*, but with a UV-absorbing red colour (Dafni *et al.*, 1990). On the other hand, birds do have red receptors, yet a number of bird-pollinated red flowers have secondary reflectance, including *Camellia japonica* (Kunitake *et al.*, 2004), *Lapageria rosea* (Martínez-Harms *et al.*, 2010) and *Clivia gardenia* (orange, South Africa), (Kiepiel and Johnson, 2014). Our findings indicate that these red flowers with secondary reflectance are less specialised for bird pollination, as they should be more readily detected by bees compared with those of pure red colour.

Although UV reflection increases the contrast of red flowers against their background and enhances the colour purity, red is not as detectable to bees as other colours, such as violet and blue (Spaethe *et al.*, 2001). This begs the question of how bee-pollination might have evolved in such flowers. Bees exhibit an innate preference for certain colours (e.g., violet and blue, Giurfa *et al.*, 1995; Lunau and Maier, 1995), but they quickly learn to prefer other colours by relating them to rewards (Menzel *et al.*, 1993). Both empirical and theoretical evidence has suggested that even pure red flowers are not invisible to bees (Chittka & Waser, 1997). Although the red floral colour of *O. confertum* is not easy to detect compared with other bee flowers, this plant offered generous nectar reward to pollinators, achieving frequent visitation. In addition, the uniqueness of this red floral colour may be advantageous to decrease the risk of heterospecific pollen transfer in the community (van der Kooi *et al.*, 2016).

In the present study, we have revealed that the UV component of reflectance contributes to enhanced attractiveness to bee pollinators for flowers with human-red colouration. Using *Onosma confertum*, we first confirmed that the UV reflection increased the chromatic contrast between red flowers and their background as perceived by bumblebees. Second, the natural foraging bumblebees showed a strong preference for the UV+ inflorescences of *O. confertum* over the UV- inflorescences in field. Third, using the human-invisible colour variation of red *Camellia* flowers, we found that the flower-naïve bumblebees showed an innate preference for UV+ targets over UV- ones. These findings suggest that the secondary reflectance, e.g., UV reflection, may be an important component in red flowers to enhance visibility to pollinators without red receptors, such as bees. This prediction can be tested by further studies with larger samples.

ACKNOWLEDGEMENTS

We thank Mr. Zhen-Dong Fang and staff from the Shangri-La Botanical Garden for help in field experiments; Dr Gao Chen for help in the colour choice experiment; Dr Klaus Lunau for help with colour analysis; Mrs Shi-Qiong Wang and Mr Zhong-Lang Wang for their kind permission to collect samples from the Kunming Botanical Garden. This study was supported by the Key Projects of the Joint Fund of the National Natural Science Foundation of China (U1802232); the Second Tibetan Plateau Scientific Expedition and Research (STEP) programme (2019QZKK0502); NSFC (31670214 to Y.N.); the Youth Innovation Promotion Association, CAS (2018427 to Y.N.); and Yunnan Ten Thousand Talents Plan Young & Elite Talents Project.

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LEGENDS TO FIGURES

Fig. 1: Bumblebee (*Bombus friseanus*) visiting the human-red flower of *Onosma confertum* (Boraginaceae) and reflectance spectra of natural, UV+ (coated with only oil substrate) and UV- (coated with sunscreen mixture and oil substrate) flowers (a). Their colour loci were mapped in the colour hexagon model (Chittka, 1992) (b); and the receptor noise-limited model (Vorobyev and Osorio, 1998) (c). The relevant region of the coordinates are detailed to show the relative positions of colour loci in more detail.

Fig. 2: Reflectance curves of the background, training and testing stimuli (cultivated varieties of *Camellia japonica*) that have been used in colour choice by flower-naïve bumble bees. Colour loci were mapped in colour hexagon units (Chittka, 1992).

Fig. 3: Chromatic contrast (a), spectral purity (b) and achromatic contrast (c) between colour loci and the background in natural, UV+ and UV- flowers. CH = colour hexagon model (Fig. 3a, left Y-axis, in colour hexagon units); RNL = receptor noise-limited model (Fig. 3a, right Y-axis, in units of Just Noticeable Differences). Bars indicate standard errors. Different letters indicate significant differences at the 0.001 level.

Fig. 4: Colour choice by experienced *Bombus friseanus* (a) and flower-naïve *B. terrestris* (b). UV+ and UV- indicate UV-reflecting and UV-absorbing red targets, respectively. The numbers in columns are sample sizes and *** indicates a significant difference at the 0.001 level.

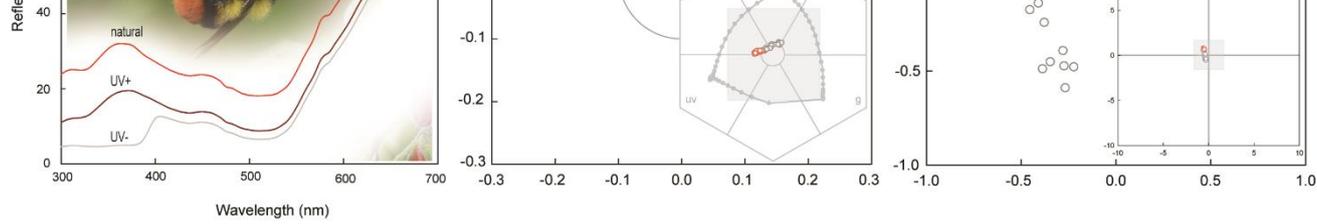


Figure 1

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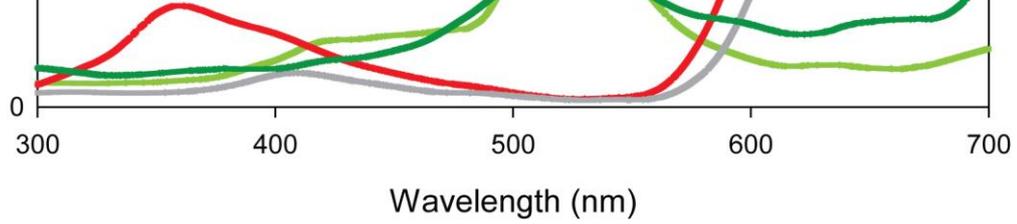


Figure 2

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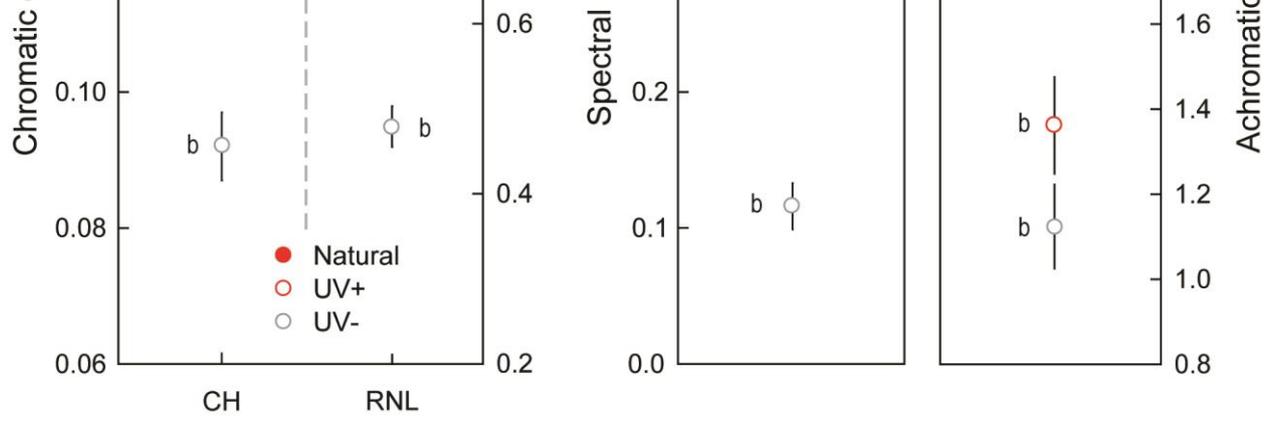


Figure 3

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Figure 4

