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Visual outdoor response of multiple wild bee species: highly selective stimulation of a single photoreceptor type by sunlight-induced fluorescence

Sujaya Rao · Oksana Ostroverkhova

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Abstract Bees have ultraviolet (UV), blue and green photoreceptor types in their compound eyes with which they locate food sources in landscapes that change continuously in cues emanating from plants and backgrounds against which they are perceived. The complexity of bee vision has been elucidated through studies examining individual species under laboratory conditions. Here, we used a bee-attractive fluorescent blue trap as a model for analyzing visual signals in operation outdoors, and across bee species. We manipulated trap color (appearance to humans under light with weak UV component) and UV-induced fluorescence emission, and aligned field capture results with bee vision models. Our studies show that the bees were attracted to traps that under solar illumination exhibited strong fluorescence emission exclusively in the blue spectral region. Through quantitative analysis, we established that strong spectral overlap of trap emittance with the photosensitivity characteristic of the blue receptor type and minimal overlap with those of the other two receptor types is the most critical property of attractive traps. A parameter has been identified which predicts the degree of attractiveness of the traps and which captures trends in the field data across wild bee species and for a diversity of backgrounds.

Keywords Wild bees · Bee vision · Selective receptor excitation · Innate behavior · Fluorescence

Abbreviations

BBF	Blue blue fluorescent
BF	Blue fluorescent
BN	Blue non-fluorescent
CBF	Clear blue fluorescent
CGF	Clear green fluorescent
CN	Clear non-fluorescent
CYF	Clear yellow fluorescent
JND	Just-noticeable-difference
RNL	Receptor noise limited
UV	Ultraviolet
YF	Yellow fluorescent

Introduction

Visual signals are critical for pollinating bees seeking appropriate flowers for food resources, namely nectar for energy and pollen for protein (Goodale et al. 2014). Some bee species are polylectic and thus use signals from a wide range of plant families, while the more specialized oligolectic and monolectic bees detect cues from fewer plant families (Milet-Pinheiro et al. 2012). The foraging period in the life cycle of a bee can extend beyond the duration of bloom in any one plant species that it visits, thus requiring an innate ability for detection and discrimination between flowers of multiple plant species in surroundings that also change in color frequently. Even flowers of the same plant species vary in color as the concentration of color pigments differs due to genetic and environmental influences (Rohde et al. 2013). Thus, bees detect multiple food resources in landscapes that differ temporally and spatially in cues

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S. Rao
Department of Crop and Soil Science, Oregon State University, 3017 ALS, Corvallis, OR 97331, USA
e-mail: sujaya@oregonstate.edu

O. Ostroverkhova (✉)
Department of Physics, Oregon State University, 301 Weniger Hall, Corvallis, OR 97331, USA
e-mail: oksana@science.oregonstate.edu

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provided by both plant communities and the backgrounds against which they are perceived.

Many aspects of color vision in bees have been thoroughly studied (Lunau 1990, 1991; Lunau and Maier 1995; Lunau et al. 1996; Chittka 1997; Vorobyev and Osorio 1998; Chittka 1999; Gumbert 2000; Spaethe et al. 2001; Dyer and Chittka 2004; Lunau et al. 2006; Chittka and Spaethe 2007; Whitney et al. 2009; Papiorek et al. 2013; Goodale et al. 2014). These studies have highlighted the complexity of bee attraction to flowers of different colors and patterns. The colors of flowers are diverse, and have evolved under selection by their pollinators. However, bee attraction to flowers cannot be categorized according to their color appearance to a human observer, because pollinators have fundamentally different visual systems to humans. Bees have a trichromatic visual system comprised of ultraviolet (UV), blue and green receptors in their compound eyes (Peitsch et al. 1992). Together, the three color receptor types comprise the inputs to a color opponent system which forms the 'color space' at the perceptual level (Chittka et al. 1994).

Most studies on bee vision have examined responses to artificial flowers or models under artificial light in laboratories, and with captive-reared honey bees, *Apis mellifera*, or bumble bees, *Bombus* spp. Very few studies have been conducted outdoors (Rohde et al. 2013) or with solitary bees (Campan and Lehrer 2002; Milet-Pinheiro et al. 2012), or examined the response of more than a single bee species. In this paper, we analyze visual signals emanating from a bee-attractive trap placed outdoors and determine the critical property of the trap that causes attraction across a wide variety of wild bee species, and in a diversity of landscapes. Our study provides a quantitative measure for enhanced innate response of wild bees to visual stimuli, which contributes to fundamental knowledge of bee color vision and can be used for designing artificial objects for drawing wild bees to targeted locations for enhancing pollination. Such insights on color vision-based innate bee responses also have potential for incorporation in plant breeding programs for sustaining or increasing attractiveness of flowers in new cultivars that are produced.

The attraction of free flying wild bees to one of the traps used in this study was noticed during an earlier study conducted to determine the response of a beetle pest to pheromone traps of different colors. In that study, the unexpected discovery was made that a particular blue fluorescent trap (Fig. 1a) did not attract the pest but drew an extraordinary abundance and diversity of wild bees (36 species in 18 genera in 5 families) even though it was set up in a large field of seedling barley with no flowers (Stephen and Rao 2007). Bees were drawn to the traps; they hit the cross vanes and fell into the container below. The non-target capture of bees belonging to four large bee families, Apidae, Colletidae, Megachilidae, and Halictidae, led to the trap being used subsequently as an effective monitoring tool for native bees in diverse habitats including cropping systems as detailed in the Supplementary Material. Examples of wild bee species captured by the trap in the USA and in Australia are given in Supplementary Material, Tables S1 and S2.

The trap served as an excellent model for our study as it exhibits color (as perceived by a human observer under light with a weak UV component) and fluorescence (under UV excitation), both of which could be manipulated. (Throughout the paper, "light with a weak UV component" will be referred to as "ambient" light. This includes indoor lighting and outdoor illumination under overcast conditions.) In addition, the trap could be placed outdoors with ease for determining the basis of attractiveness of the trap to free flying wild bees. In this study, we first created colored and colorless (where "colorless" is defined as "appeared colorless to humans under ambient light") traps with varying intensity and spectra of fluorescence emission and evaluated bee responses to such traps. Then, we analyzed the spectral attributes of the attractive and non-attractive traps and related them to existing bee vision models. Finally, we identified a parameter which predicts the degree of attractiveness of the traps across wild bee species, and for a diversity of backgrounds.

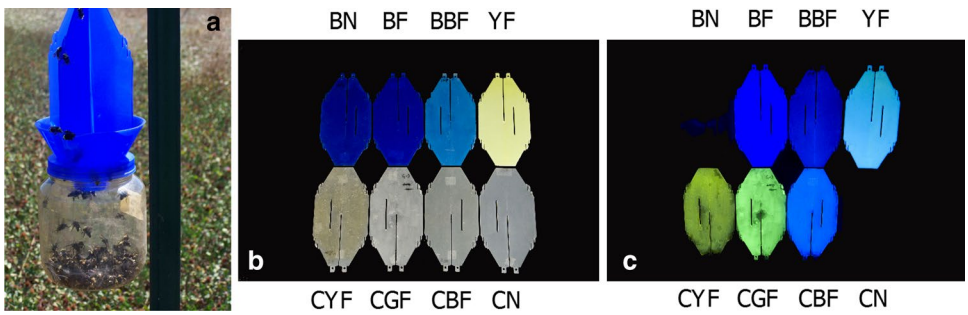

Fig. 1 **a** *Blue* fluorescent (BF) trap. Vanes from traps used in our studies under ambient light (**b**) and UV light (**c**). *Top row* colored vanes BN, BF, BBF, and YF. *Bottom row* colorless vanes CYF, CGF, CBF, and CN (*control*). No fluorescence emission was observed from the BN and CN vanes

Table 1 Color (as perceived by a human observer under ambient light) and fluorescence emission of traps used in field studies

Trap	Color under ambient light	Fluorescence
BF (blue fluorescent)	Blue	~400–500 nm (430 nm)
BN (blue non-fluorescent)	Blue	None
YF (yellow fluorescent)	Yellow	~400–570 nm (430 nm)
CBF (clear blue fluorescent	Clear	~400–520 nm (435 nm)
CGF (clear green fluorescent)	Clear	~470–600 nm (505 nm)
CYF (clear yellow fluorescent)	Clear	~490–600 nm (535 nm)
BBF (blue blue fluorescent)	Blue	~400–520 nm (450 nm)
CN (clear non-fluorescent)	Clear	None

Spectral range of fluorescence emission under UV light excitation is indicated. Wavelength of peak fluorescence emission is shown in parenthesis

Materials and methods

Two field studies were conducted in the state of Oregon in western USA in a landscape dominated by agricultural fields (Figs. S1, S2). The traps used in the study consisted of two polypropylene cross vanes (24 × 13 cm; 3 mm thick) inserted into a polypropylene screw cap funnel placed over a clear plastic collecting jar, 15 cm diameter and 15 cm height (Fig. 1a). No odor was added to the traps.

Table 1 summarizes properties of the traps used in our studies. Clear non-fluorescent cross vanes and screw cap funnels (CN) served as the controls. CN, BF, YF and BN were obtained from Spring Star (Woodinville, WA, USA). For the remaining traps (CBF, CYF, CGF, and BBF), we painted clear vanes and screw cap funnels (=CN) with fluorescent paints (Risk Reactor, Inc.) to obtain the range of properties listed in Table 1. Appearances of vanes under ambient and under UV light are presented in Fig. 1b and c, respectively.

Measurements of optical properties of traps

Total spectral emittance for all traps was obtained by exciting the vane with simulated sunlight under AM 1.5G conditions, typically used in characterization of solar cells (solar simulator Oriel 96000 with AM 1.5G filter) (Ostroverkhova 2013), and measuring a signal emitted from the vane's front surface with a calibrated fiber-coupled spectrometer (Ocean Optics USB2000). (Here the "AM" stands for "air mass" defined as AM = 1/cos (θ), where θ is the zenith angle, and "G" stands for "global". The "AM 1.5G" represents solar illumination of a tilted surface (37°) with $\theta = 48°$ at light intensity 963 W/m^2. These conditions correspond to average solar illumination conditions in the USA.) Diffuse reflectance from the vanes at wavelengths above 400 nm was measured in the same geometry using either a halogen lamp or the solar simulator with a 395 nm long-pass filter, to ensure that the signal is not affected by UV-induced fluorescence. UV reflectance at 300–400 nm was measured using a Xe lamp, double monochromator, and a calibrated photodetector. Spectralon 50 and 100 % reflectance standards from LabSphere, Inc. were used as references with known diffuse reflectance properties. Fluorescence in the vanes was excited with a UV part of solar AM 1.5G radiation (280–400 nm) (solar simulator with AM1.5G filter, dichroic mirror Oriel 81045 and a UV band-pass filter Oriel 81046) and measured in the same geometry as reflectance (Platt et al. 2009, 2011; Paudel et al. 2014). Similar measurements of fluorescence were performed using UV lamp (360–400 nm) and outdoor sunlight with a UV band-pass filter (Oriel 81046) as excitation sources and yielded identical emission spectra.

Field studies

In each study, the traps were set up on private lands after consultation with the farmer. None of the wild bees trapped were endangered or protected, and no specific permissions were required.

Influence of sunlight-induced fluorescence on wild bee response

To determine the impact of sunlight-induced fluorescence on bee captures, we compared wild bee captures in traps with the same blue pigment with and without fluorescent additives (BF and BN, respectively). CN traps were used as the control. Optical properties of BF, BN, and CN traps are shown in Figs. 2 and 3. Strong fluorescence emission in the blue spectral region, peaked at ~430 nm, was observed in the BF traps (Fig. 2a) but was absent in the BN and the CN traps. In the visible spectral range, the reflection properties of the blue BF and BN traps were similar (Fig. 2b), whereas in the UV spectral range (300–400 nm), the BF trap was less reflective than the BN trap, due to absorption of UV light by fluorescent additives. Because of the efficient UV light absorption and strong fluorescence in the blue, the overall photon flux emitted from the BF trap in the blue (UV) spectral region was considerably enhanced (reduced) as compared to that from the BN trap (Figs. 3a and S3).

The traps were set up along the margin of fields of red clover (*Trifolium pratense* L.) seed crops during bloom as a randomized block design with three replications. Treatments were separated by at least 7 m (e.g., Figs. S1, S2), and blocks (=independent replicates) were separated by >30 m. The experiment was repeated for four consecutive days. On each day, the traps were set up at 8 a.m. and wild bees were collected at 5 p.m. the same day, preserved, and subsequently identified.

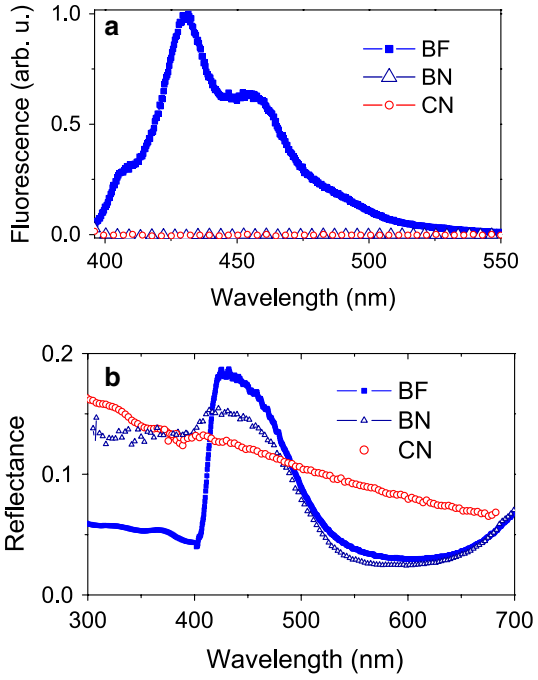

Fig. 2 **a** Fluorescence under excitation with the UV part of solar AM 1.5G radiation and **b** reflectance spectra of the BF, BN, and CN vanes used in all field studies. No fluorescence from the BN and CN vanes was observed under these conditions

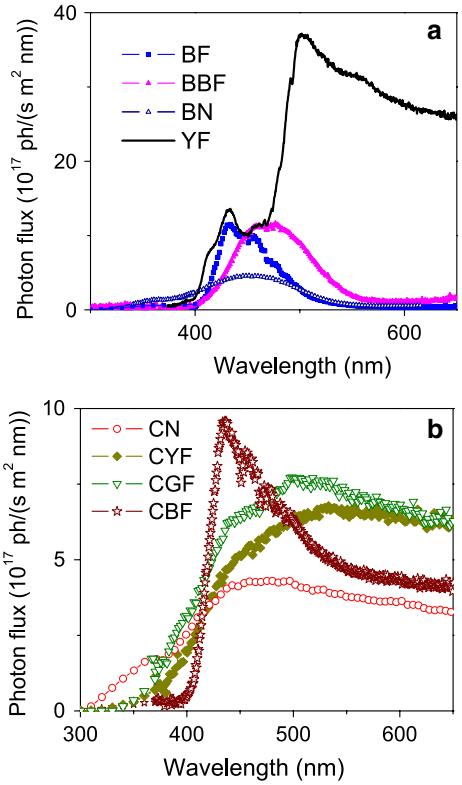

Fig. 3 Total photon flux emitted from the BF, BN, BBF, and YF vanes (**a**) and from the CBF, CGF, CYF, and CN vanes (**b**) under solar AM 1.5G illumination

Influence of fluorescence emission spectra and intensity on wild bee response

Three trials were conducted over different periods to determine the impact of UV-induced fluorescence with different spectra and intensity. In the first trial, we repeated the first study described above but added CBF, CYF, CGF and BBF traps. The experiment was set up as a randomized block design with four replicates (=separate agricultural fields). The treatments (traps) were set up at least 7 m apart along the margins of red clover fields separated by >800 m. The traps were set up and emptied over four consecutive days from 8 a.m. to 5 p.m. the following day, preserved, and subsequently identified. In the second and third field trials, traps with YF vanes were added, and the experiment was set up for 1 day each.

Honey bees were present in the traps in both field studies but they were excluded from the study as they originated primarily from hives that were placed by farmers for pollination of the red clover seed crops. Honey bees are not native to the US but have been 'domesticated' or 'managed' for crop pollination. No other 'managed' bee species was used for crop pollination in the region and hence trap captures that were analyzed represented wild bees alone.

Optical properties of traps used in these experiments are summarized in Table 1 and shown in Fig. S4; total photon flux emitted by the traps under solar illumination is presented in Fig. 3. All fluorescent traps had a low emittance in the UV spectral range, due to efficient absorption of UV light by the fluorescent dye. The yellow colored YF (Fig. 1b) had a broad reflectance spectrum and strong fluorescence emission in the blue and green spectral regions (Table 1; Fig. S4), resulting in the overall emitted photon flux peaked at ~500 nm under solar illumination (Fig. 3a). Fluorescence emission in the CBF peaked at ~435 nm, in the CGF at ~505 nm, and in the CYF at ~535 nm (Fig. S4a). Under the same excitation conditions (i.e., by the UV part of the solar spectrum as described above), the CBF exhibited highest fluorescence intensity among the colorless traps CBF, CGF, and CYF (which resulted in a large enhancement of the overall CBF emittance in the blue spectral range under solar illumination, Figs. 3b, S3); however, it was lower than that of the BF (Fig. S4a). Reflectance properties of the CBF, CGF, and CYF traps were featureless, and similar to each other, as expected from colorless objects (Fig. S4b). The BBF had a peak reflectance in the blue spectral region and weak fluorescence emission that peaked at ~450 nm, resulting in an overall emitted photon flux peaked at ~470 nm under solar illumination (Fig. 3a).

Data analysis

For both field studies, bee captures in traps were averaged per day, transformed using sqrt (X) (first study) or log ($X + 1$) (all three trials in the second study) to stabilize variance, and submitted to an analysis of variance (R program). For the second study, bees trapped were separated as 'bumble bees' and 'other wild bees' for statistical analysis. Tukey's multiple comparison procedure was used to determine significant differences amongst the treatment with $\alpha = 0.05$.

Spectral analysis

The receptor-specific contrast P_i of the traps with respect to the average background was calculated as follows (Chittka et al. 1994):

$$P_i = R_i \int_{300}^{700} S_i(\lambda) I_{\text{total}}(\lambda) \mathrm{d}\lambda \tag{1}$$

where the index i corresponds to the UV, blue, or green receptor (i.e., i = UV, B, G), λ is the wavelength of light (in nanometers), and S_i is the spectral sensitivity function of the ith receptor (Papiorek et al. 2013). Receptor photosensitivity characteristics were not available for the wild North American bees captured by our traps and hence we used, as comparative analytical standards, those of the following four European bees: *Bombus terrestris* (L.) (Apidae), *Anthophora acervorum* (L.) (Apidae), *Osmia rufa* L. (Megachilidae), and *Andrena florea* (Fabr.) (Andrenidae) (Peitsch et al. 1992). These were chosen as all four genera have been caught in the traps under study. The I_{total} is the total photon flux calculated from spectral emittance of the vane (Fig. 3), which includes components both due to reflectance of the incident sunlight and due to fluorescence emission excited by the UV part of incident sunlight. R_i is the sensitivity factor of the ith receptor given by (Chittka et al. 1994):

$$R_i = 1 \Big/ \int_{300}^{700} R_\text{B}(\lambda) S_i(\lambda) I_{\text{sun}}(\lambda) \mathrm{d}\lambda,$$

where R_B is the spectral reflectance of the background and I_{sun} is the photon flux obtained from spectral irradiance of solar illumination, for which we assumed AM 1.5G conditions (Fig. S3). Since the background in our field experiments was diverse, the parameters P_i were calculated for two prevailing adaptation backgrounds, green vegetation and dry grass (Fig. S1) (Peitsch et al. 1992). Based on the definition of P_i, for any given adaptation background $P_i = 1$.

The phototransduction of photoreceptor absorption (P) into receptor excitation (E) was calculated using (Chittka and Menzel 1992; Chittka et al. 1994):

$$E_i = P_i/(P_i + 1), \tag{2}$$

where E_i is the ith receptor excitation ($E_i = 0.5$ when the ith receptor receives a signal from the object equal to that from the background). Color contrast was examined using a color hexagon model of Ref. (Chittka and Menzel 1992), a two-dimensional description of the three-dimensional receptor space, to describe colors as seen by a bee. Within the hexagon, three excitation values E_i of Eq. (2) corresponding to the three receptors (i = UV, B, or G) are combined to yield a data point (x, y) representing the color of the object in the bee color space, with x and y coordinates defined as:

$$x = \frac{\sqrt{3}}{2}(E_\text{G} - E_\text{UV}); \quad y = E_\text{B} - \frac{1}{2}(E_\text{G} + E_\text{UV}). \tag{3}$$

In such description, the origin at $x = y = 0$ represents a color perceived by the bees as a background to which they are adapted. The color contrast is defined as the distance between the origin (0, 0) and the (x, y) point describing a vane color on the color hexagon, i.e., $\sqrt{x^2 + y^2}$.

Chromatic contrasts were also calculated using the receptor noise limited (RNL) model (Vorobyev and Osorio 1998). Chromatic contrasts (ΔS), in just-noticeable-difference (JND) units, were calculated using

$$\Delta S = \sqrt{\frac{\omega_\text{UV}^2 (f_\text{G} - f_\text{B})^2 + \omega_\text{B}^2 (f_\text{G} - f_\text{UV})^2 + \omega_\text{G}^2 (f_\text{UV} - f_\text{B})^2}{\omega_\text{UV}^2 \omega_\text{B}^2 + \omega_\text{UV}^2 \omega_\text{G}^2 + \omega_\text{B}^2 \omega_\text{G}^2}} \tag{4}$$

Here f_i is the difference in receptor signal between stimulus and background, $f_i = \ln(P_i)$ ($f_i = 0$ for background), and ω_i is the standard deviation of noise. In our analysis, we used values $\omega_\text{UV} = 1.3$, $\omega_\text{B} = 0.9$ and $\omega_\text{G} = 0.9$ for the bumble bee (Skorupski and Chittka 2010).

Results

The average numbers of wild bees captured in the traps in the first field study are presented in Fig. 4.

The analysis of variance indicated that the traps differed significantly in captures of wild bees ($F = 112.9$, $df = 2.6$; $P < 0.0001$). Based on the Tukey multiple comparison test, there were significantly more bees captured in traps with blue fluorescent vanes (BF) compared with those that lacked fluorescence (BN and CN) ($P < 0.0001$). The non-fluorescent traps (BN and CN) did not differ significantly in trap captures ($P < 0.3$).

The blue traps BF and BN had similar reflection properties in the visible spectral range (Fig. 2b) and looked the same to us under ambient light (Figs. 1b, S1). However, for both groups of wild bees, captures in BF were significantly

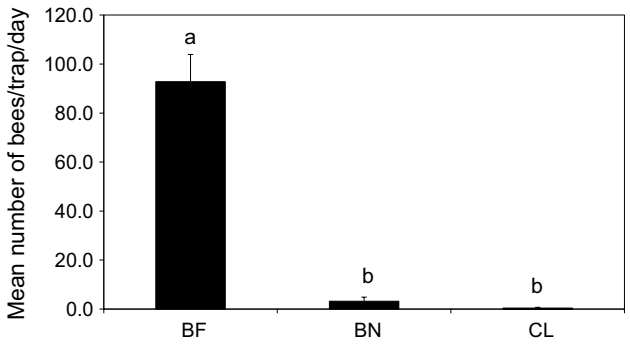

Fig. 4 The mean number of wild bee captures per trap per day (*bars*) in the first field study. *Error bars* represent standard error. Different *letters* above *bars* indicate statistically significant differences (Tukey's multiple comparison test after analysis of variance)

higher than those in BN which indicates that appearance of the trap under ambient light is not the dominant factor influencing attraction of wild bees to the BF traps. Instead, it is sunlight-induced fluorescence (Fig. 2a) that plays a critical role in the dramatic difference in bee responses to BF versus BN traps.

The average numbers of bumble bees and other bees captured in the traps across the three trials in the second field study are presented in Fig. 5.

Bumble bees The analysis of variance indicated that the traps differed significantly in captures of bumble bees in all trials (Trial 1: $F = 11.9$, $df = 6, 21$; $P < 0.0001$; Trial 2: $F = 11.1$, $df = 7, 24$; $P < 0.0001$; Trial 3: $F = 14.8$, $df = 7, 24$; $P < 0.0001$). Based on the Tukey's multiple comparison test, there were significantly more bumble bee captures in BF traps compared with any other trap in all three trials ($P < 0.0001$). Captures in the CBF (which appeared colorless to us under ambient light, Figs. 1b, S2) differed significantly from CN captures in trial three, but not in trials one and two. Bumble bee captures in the remaining traps (BBF, BN, CYF, YF, CGF and CN) did not differ significantly across the trials.

Other wild bees The analysis of variance indicated that the traps differed significantly in captures of wild bees other than bumble bees (Trial 1: $F = 21.1$, $df = 6.\ 21$; $P < 0.0001$; Trial 2: $F = 10.4$, $df = 7.\ 24$; $P < 0.0001$; Trial 3: $F = 31.6$, $df = 7.\ 24$; $P < 0.0001$). While there were higher numbers of bees in BF traps compared to all other traps, based on the Tukey's multiple comparison test, the differences compared with CBF were only significant in trial three but not in trials one and two. There were more bees in the CBF traps compared with captures in the CGF and CN (in all three trials), YF (both trials two and three), and BN and CYF in trials one and three. The BBF traps captured significantly more bees as compared to the CGF and CN traps in trials one and three and, additionally, to the CYF in trial one. The non-fluorescent blue trap BN did not exhibit statistically significant differences in bee captures from the control trap CN in any of the trials, in agreement with observations of the first field study.

Discussion

Wild bee response predictions using bee vision models

Our field studies conclusively demonstrated that among the traps studied the blue fluorescent trap BF was most attractive for wild bees in all trials. The colorless trap CBF, which exhibited fluorescence emission in the blue spectral region similar to that of the BF, was the next best trap that attracted significant number of wild bees, statistically different from those in the control trap CN in one of the three trials in the case of bumble bees and in all three trials for other wild bees. These were followed by the blue, relatively weakly fluorescent trap BBF, which captured significantly more wild bees (other than bumble bees) than the control trap CN in two out of three trials. Interestingly, YF traps, which had strong fluorescence in both blue and green spectral regions, as well as CGF and CYF traps with fluorescence emission in the green and yellow spectral regions, respectively, were not attractive. Finally, the BN trap that had the same human-perceived color under ambient light as the BF (Figs. 1b, S1), but did not have any fluorescence emission, was not attractive in any of the trials.

Our findings can be summarized as follows: (1) in the presence of strong fluorescence emission under excitation with UV light, the perceived color (i.e., appearance of the trap to humans under ambient light) is not an important factor for bee attraction; (2) fluorescence intensity is important: the bees were attracted to the traps with strongest fluorescence emission; and (3) fluorescence spectra are important: for example, the bees were attracted to the traps with strong and narrow fluorescence emission in the blue, but not to the traps with strong and broad-band fluorescence emission which covered both blue and green spectral regions.

Next, we attempted to align results of our field studies with previously developed bee vision models (Chittka and Menzel 1992; Vorobyev and Osorio 1998). Since in our studies we dealt with wild bees, with unknown flight pattern, the target detection mechanism was not known a priori, and thus we analyzed both green and color contrast characteristics of our vanes (Spaethe et al. 2001). For that, using the spectral emittance data obtained from our traps under solar illumination and receptor photosensitivity characteristics for four bee species, we calculated receptor-specific contrasts P_i (i = UV, B, G) for each trap as described in "Materials and methods". The green contrast P_G is shown in Fig. 6a for the case of bumble bee, and

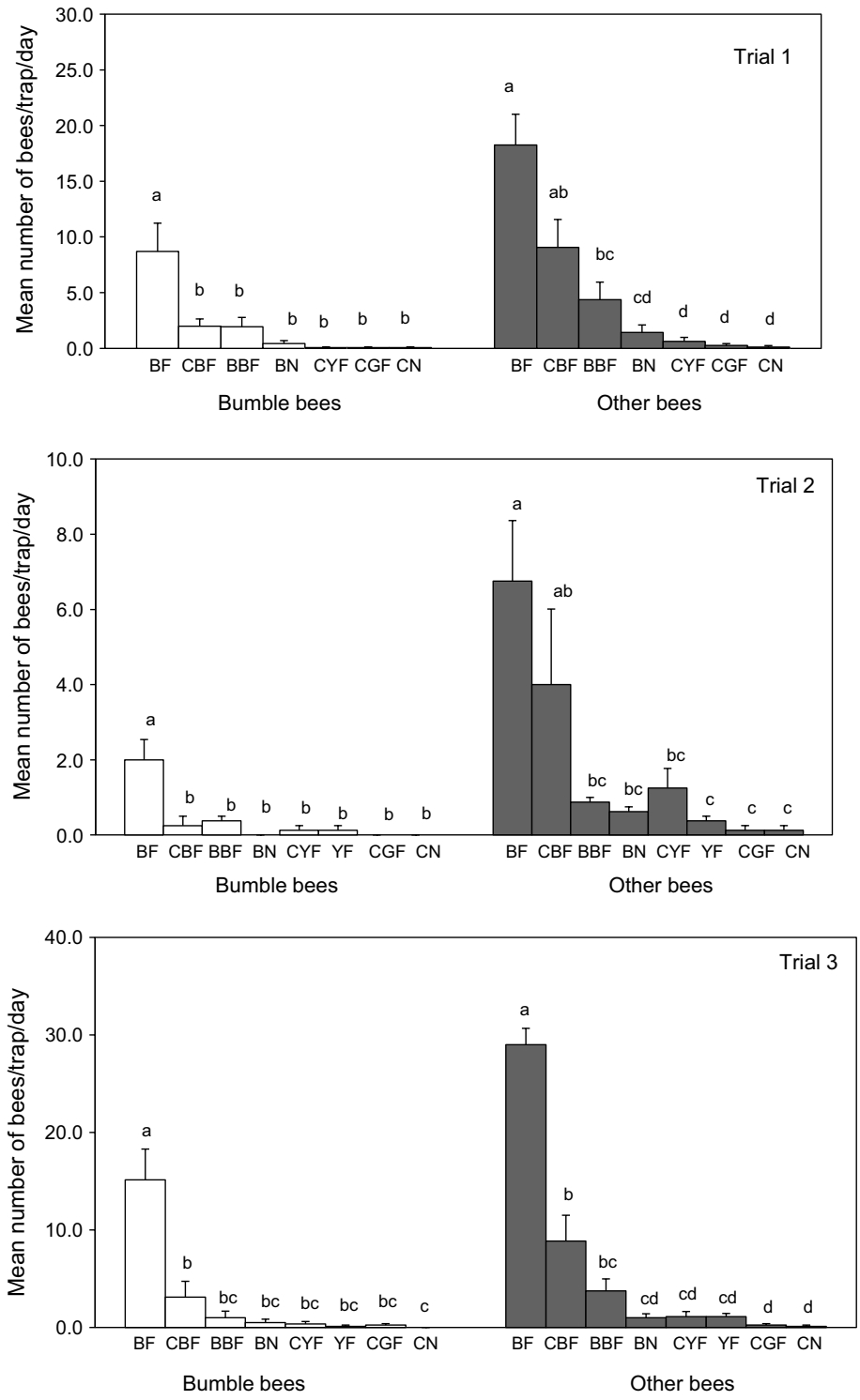

Fig. 5 The mean number of bee captures per trap per day (*bars*) in the second field study. Bumble bee and all other wild bee species captures (see, for example, Table S1) are shown separately. *Error bars* represent standard error. Different *letters* above *bars* within each group indicate statistically significant differences (Tukey's multiple comparison test after analysis of variance)

assuming green vegetation adaptation background. The YF traps had the highest green contrast of all traps. Therefore, if the attraction of bees to our traps was based on the green contrast, YF would have been most attractive. Furthermore, as the attractive BF traps had a considerably lower green contrast P_G than most other traps, they would be expected to be less attractive than other traps. The trends of Fig. 6a were similar for all four bee species, and for both adaptation backgrounds studied. As such trends clearly contradict results of our field studies of Fig. 5, we conclude that the green contrast is not a major factor in wild bee response to our traps.

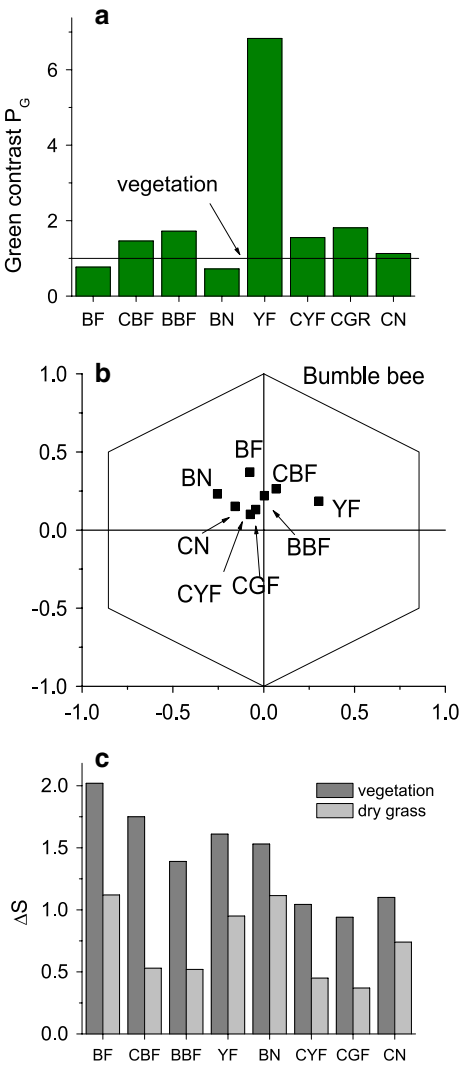

Fig. 6 **a** *Green* contrast P_G of Eq. (1) for all traps under solar illumination. **b** Representation of traps in bee *color* space using Eq. (2). Parameters in **a**, **b** were calculated for the bumble bee, and assuming *green* vegetation adaptation background. **c** Chromatic contrast ΔS calculated using Eq. (4) for all traps for the bumble bee, and assuming *green* vegetation and dry grass adaptation backgrounds

Then, we considered color vision of the bee and obtained the "color" of each trap in the bee color space using Eq. (3) shown in Fig. 6b for the case of the bumble bee, and assuming green vegetation adaptation background. According to this model, most of our traps are in the "Blue" and "UV-Blue" parts of the bee color space, with the exception of the YF, which is in the "Blue–Green" part (Chittka et al. 1994). It has been previously reported that untrained bumble bees favor targets with higher color contrast, ignoring dominant wavelength (Lunau 1992). However, if this was the case in our field studies, the non-attractive BN and YF traps would be as attractive to the wild bees as the attractive BF, as their color contrast values were comparable (0.35, 0.36, and 0.38, respectively, Figs. 6b, S5a). This prediction clearly contradicts the results of our field studies of Fig. 5. Similarly, we found that spectral purity, defined as the color contrast of an object relative to that of the corresponding dominant wavelength (Lunau 1990; Rohde et al. 2013), is not a good predictor for observed bee attraction to the BF traps, but not to the BN or YF traps (Fig. S5b).

Thus, it is necessary to obtain a parameter that would better describe the trends in bee captures by our traps. Additional challenge presented by the nature of our field studies, which were carried outdoors in diverse habitats with wild bees, is that the sought parameter has to predict the correct trends that hold for a wide variety of wild bee species and for different adaptation backgrounds.

Next, we considered an alternative model of bee color vision and calculated chromatic contrasts ΔS for each trap using the RNL model of Eq. (4) as described in "Materials and methods". Figure 6c shows ΔS obtained for various traps, at two adaptation backgrounds, for the bumble bee. For the green vegetation background, the ΔS value exhibited a trend comparable to results of our field studies: it had a highest value for the most attractive BF trap, followed by that for the next best trap, CBF. However, for the dry grass adaptation background, completely different trends were observed. For example, the chromatic contrast ΔS for the attractive BF trap was similar to that of the non-attractive BN trap. Thus, if the wild bee attraction to traps were related to the value of the chromatic contrast ΔS, similar bee captures would be expected in the BF and BN traps, in a strong disagreement with the experimental data of Figs. 4 and 5. Further testing of predictions of the RNL model against results of our field study was not possible since the receptor noise values ω_i of Eq. (4) for wild bee species other than bumble bees were not available from the literature.

To address the difficulty of dealing with a great variety of wild bee species and varied backgrounds we needed a parameter as independent of specific bee vision models as possible, which only requires knowledge of spectral characteristics of the visual stimulus and of receptor photosensitivity characteristics.

Excitation of the blue receptor type, as exclusive as possible, is the critical factor

Based on the spectral characteristics of the BF and CBF traps which were most attractive to the bees, we introduce a relative blue receptor contrast

$$\boldsymbol{p} = P_B/(P_B + P_G + P_{UV}), \quad (5)$$

where P_i is the receptor-specific contrast given by Eq. (1) as described in "Materials and methods". The relative blue receptor contrast $\boldsymbol{p}$ quantifies the degree to which the blue

receptor excitation is *exclusive*. Note that this notion is considerably different from the spectral purity defined above, and high spectral purity is not a sufficient condition for obtaining high value of $\boldsymbol{p}$ of Eq. (5). On the other hand, $\boldsymbol{p}$ does not provide a direct measure of the monochromaticity of the stimulus, instead quantifying the spectral overlap between the visual stimulus and the receptor photosensitivity characteristics. With the definition of Eq. (5), the highest theoretically achievable value of $\boldsymbol{p}$ is 1, at which only the blue receptor type, and not the UV or green receptor types of the bee, is excited. However, because of the spectral overlap of photosensitivity characteristics of the three receptor types, $\boldsymbol{p} = 1$ is unattainable and thus $\boldsymbol{p} < 1$. For the adaptation background ($P_i = 1$), $\boldsymbol{p} = 0.33$.

The parameter $\boldsymbol{p}$ for each trap was calculated using the P_i values obtained with photoreceptor sensitivities of four different bee species, and for two adaptation backgrounds. Figure 7 (left axis) shows the parameter $\boldsymbol{p}$ obtained for each trap relative to that for the control trap CN, for bumble bees, assuming green vegetation and dry grass adaptation backgrounds. For both backgrounds, the BF trap had the highest $\boldsymbol{p}$ value of all traps, followed by the CBF and BBF traps. This correlates well with the results of our field studies. To better illustrate such correlation, also included in Fig. 7 (right axis) are the average numbers of bee captures for each trap from trial three of the second field study (Fig. 5c), normalized by those in the BF trap: clearly, high bee captures correspond to high values of the relative blue receptor contrast $\boldsymbol{p}$ of Eq. (5).

Next, we tested the robustness of this approach against different bee species. For all bee species studied (*Bombus*, *Osmia, Andrena,* and *Anthophora*), the parameter $\boldsymbol{p}$ of the most attractive BF trap was considerably higher than that of any other trap (Fig. S6). For all species except for *Osmia*, the $\boldsymbol{p}$ values for the next best CBF trap were next highest. For all species except for *Andrena,* the $\boldsymbol{p}$ values for the BBF trap, which attracted significantly more wild bees (other than bumble bees) in two out of three trials than the control trap CN, were higher than those for all remaining traps (Fig. S6). Based on these observations, if we assume that the value of $\boldsymbol{p}$ predicted the degree of wild bee attraction to a particular trap, the BF would be most attractive for most bee species and CBF and BBF would be more attractive than the remaining traps to some bee species but not the others. This is fully consistent with the trends observed in our field studies.

Finally, we studied the quantitative relationship between the parameter $\boldsymbol{p}$ and the number of bee captures. For this, we chose field data from trial three of the second field study (Fig. 5c) as it had the highest overall number of the captures, which minimized the error. Figure 8 shows the average number of bees (per day per trap) for each trap, normalized by that in the BF trap, plotted against the

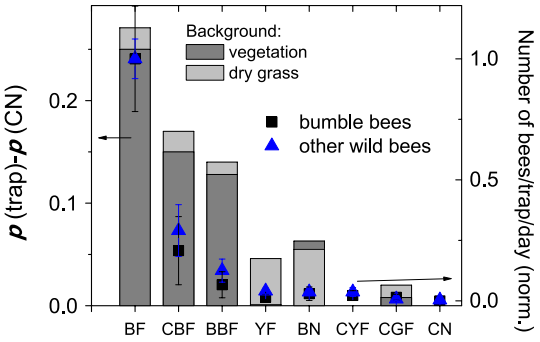

Fig. 7 The relative *blue* receptor contrast $\boldsymbol{p}$ of Eq. (5) for each trap (*bars*) relative to that of the control trap CN for the bumble bee, assuming *green* vegetation and dry grass adaptation backgrounds (*left axis*). The mean number of bee captures per trap per day (data points), normalized by that in the BF trap, in trial three of the second field study (*right axis*). Bumble bee (*squares*) and all other wild bee species (*triangles*) captures are shown separately. *Error bars* represent standard error

parameter $\boldsymbol{p}$ calculated for that particular trap. In particular, in Fig. 8a, field data for the bumble bees in Fig. 5c were used, and values of $\boldsymbol{p}$ for the traps were calculated using bumble bee receptor photosensitivity characteristics, and assuming green vegetation and dry grass adaptation backgrounds. In Fig. 8b, field data for "other bees" in Fig. 5c were used, and the values of $\boldsymbol{p}$ for the traps were calculated using *Osmia*, *Andrena*, or *Anthophora* receptor photosensitivity characteristics, and assuming green vegetation background. It is seen in Fig. 8 that regardless of the bee species or the background, the same trend persists: at values higher than a certain value of the relative blue receptor contrast $\boldsymbol{p}$, which we denote $\boldsymbol{p_0}$, any further increase in this parameter is expected to result in a dramatic increase in the number of captured bees. To obtain quantitative estimates of $\boldsymbol{p_0}$ and of the rate of the increase in the bee captures with increased value of $\boldsymbol{p}$, $\Delta\boldsymbol{p}$, we fitted the data of Fig. 8 with a single exponential function ($\sim\exp[(\boldsymbol{p} - \boldsymbol{p_0})/\Delta\boldsymbol{p}]$). The fits revealed that the values of $\boldsymbol{p_0}$ varied between 0.43 and 0.7, depending on the bee species, for the case of green vegetation background. Also, they were considerably lower for the dry grass, as compared to green vegetation, adaptation background. For example, $\boldsymbol{p_0}$ of 0.39 was obtained for a bumble bee assuming dry grass adaptation background, as compared to 0.7 in the case of the green vegetation background. The parameter $\Delta\boldsymbol{p}$ yielded similar values of 0.058 ± 0.008 and 0.060 ± 0.006 for a bumble bee, assuming green vegetation and dry grass backgrounds, respectively. For other wild bees, values of $\Delta\boldsymbol{p} = 0.07 \pm 0.01$ for *Anthophora*, 0.06 ± 0.02 for *Andrena*, and 0.030 ± 0.005 for *Osmia* were obtained (Fig. 8b). This indicates that for a variety of wild bee species, when $\boldsymbol{p}$ is higher than $\boldsymbol{p}_0$, each subsequent increase in $\boldsymbol{p}$ by 0.03–0.07 would increase bee captures

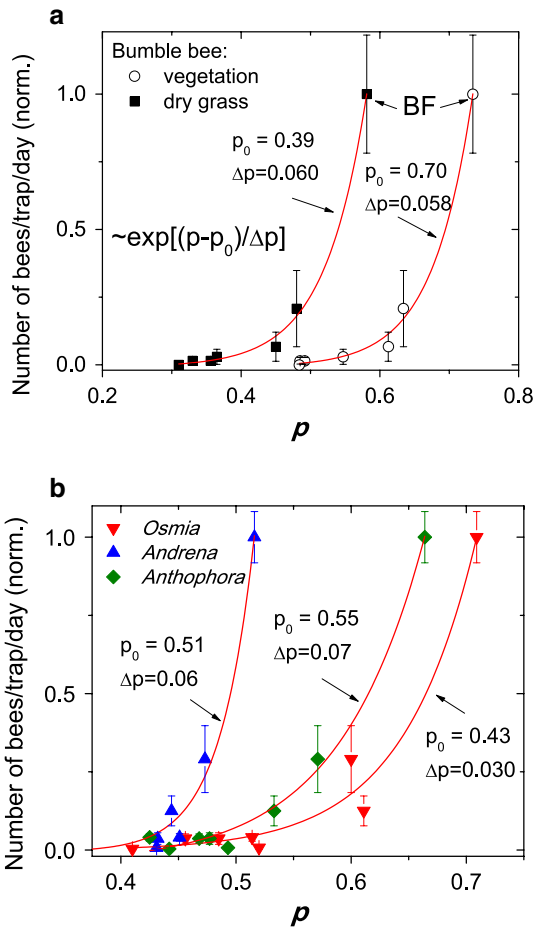

Fig. 8 The mean number of bee captures per trap per day (in trial three of the second field study), normalized by that in the BF trap, plotted as a function of $\boldsymbol{p}$ of Eq. (5) for: **a** bumble bee, assuming *green* vegetation (*open circles*) and dry grass (*solid squares*) adaptation background and **b** *Osmia* (*down triangles*), *Andrena* (*up triangles*), and *Anthophora* (*diamonds*) assuming *green* vegetation adaptation background. *Error bars* represent standard error. Fits with a single exponential function (~exp[$(\boldsymbol{p} - \boldsymbol{p}_0)/\Delta\boldsymbol{p}$]) (*red lines*) and fit parameters are also shown

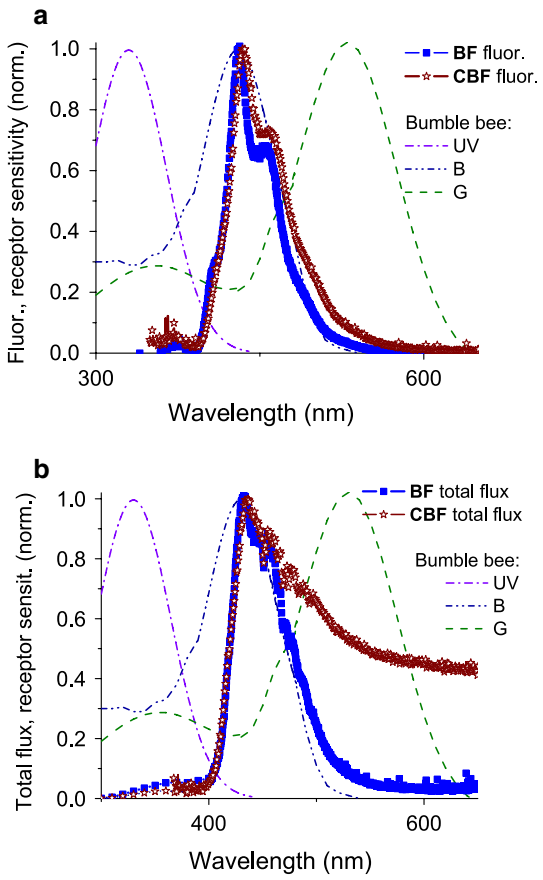

Fig. 9 Bumble bee receptor (UV, B, G) photosensitivity characteristics superimposed with: **a** fluorescence emission and **b** total photon flux of the BF and CBF traps under solar illumination. All data were normalized by their corresponding peak values

by a factor of $e \approx 2.71$. This could be used as a guide for design of agricultural and analytical tools used in manipulations and assessments of wild bee population.

Fluorescence facilitates highly selective excitation of a single receptor type

Spectral purity (color saturation) of visual stimulus has been recognized to be an important characteristic that attract bees towards particular targets. Generally, unequal stimulation of the three types of receptors, such as the case in our most attractive traps, generates spectral purity. Preferential excitation of one or two out of three photoreceptor types, obtained with artificial flowers with strong reflectance at particular spectral ranges, has been previously identified to play an important role in innate color preferences of bumble bees (Lunau and Maier 1995). Lunau (1990) showed that naïve bumble bee workers oriented themselves at a gradient of centripetally increasing spectral purity. From a distance, approach to flower models was guided by the spectral purity of the flower corolla color. At close range the bumble bees selected that area of the flower model which was highest in spectral purity (the artificial guide for antennal contact which occurred when the workers were still in flight) (Lunau 1991). By comparing the spectral reflectance in different parts of the flower and a representation of the color loci in the bee color space, Lunau (1992) demonstrated the presence of a gradient of centripetally increasing spectral purity in flower patterns as perceived by a bumble bee. More recently, Rohde et al. (2013) presented trained bumble bees with choices of artificial flowers with small shifts towards lower and higher spectral purity as compared to that of the training targets. They observed that bees preferred artificial flowers with the higher spectral purity. In tests where bumble bees were

presented a centripetal spectral purity pattern, bees chose the most spectrally pure area for the first antennal contact.

Our analysis revealed that the relative blue contrast $\boldsymbol{p}$ of Eq. (5) was the only parameter that correlated well with the observed bee captures among all parameters tested including the spectral purity, and that relatively high values of $\boldsymbol{p}$ were necessary to achieve strong bee attraction to the trap ($\boldsymbol{p} > \boldsymbol{p_0}$, where $\boldsymbol{p_0}$ depends on the adaptation background and on the bee species, Fig. 8). Such high values of $\boldsymbol{p}$ were achieved in the traps that were highly fluorescent exclusively in the blue spectral region. In particular, strong and relatively narrow fluorescence emission spectrum, when imposed on the broad reflectance spectra, increases $\boldsymbol{p}$ when the emission spectrum considerably overlaps with photosensitivity characteristic of one receptor type and not the others (Fig. 9). For example, the fluorescence of the BF has a strong overlap with photosensitivity of the blue receptor type and minimal possible overlap with those of the UV and green receptor types (Fig. 9a). It is imposed on the BF reflectance spectrum which also has a much stronger component within the blue receptor type photosensitivity characteristic as compared to green and UV receptor types, resulting in the total BF trap emittance under solar illumination exciting the blue receptor nearly as exclusively as possible (Fig. 9b). The CBF fluorescence also has a strong overlap with the blue receptor photosensitivity (Fig. 9a), but because of broad reflectance spectrum it has a sizeable overlap with the green receptor photosensitivity (Fig. 9b) as well. This lowers $\boldsymbol{p}$ and makes the CBF trap less attractive to the bees as compared to the BF, as observed in our field studies (Fig. 5). Note that the YF trap, which had the highest overall emittance in the blue spectral region (leading to the highest value of the blue receptor contrast P_B) of all traps studied (Fig. 3a), had a considerably lower $\boldsymbol{p}$ than that of the BF or CBF traps (Figs. 7, S6). This is because of strong emittance in the green spectral region as well, which results in a high green contrast P_G (Fig. 6a) that lowers $\boldsymbol{p}$ and renders the YF trap unattractive to the bees (Fig. 5b, c).

The presence of fluorescence emission from flowers, insects, and other natural organisms has been well-documented, and its contribution to behavioral patterns has been a subject of intensive research (Fasel et al. 1997; Pearn et al. 2001; Gandia-Herrero et al. 2005; Andrews et al. 2007). Of relevance to our studies is that the sunlight-induced fluorescence in artificial and natural objects that matches spectral photosensitivity characteristic of one receptor type as closely as possible can be an efficient way of achieving highly selective excitation of that receptor type. Certain dyes and molecular assemblies, such as J-aggregates, exhibit narrow fluorescence spectra; these can be utilized in artificial objects (such as traps) to achieve large parameter $\boldsymbol{p}$ without the need for powered light sources such as light-emitting diodes; they also exist in nature (Saikin et al. 2013) and may have a function related to this property. Furthermore, in most fluorescent molecules spectrum of fluorescence emission does not depend on the wavelength of excitation within its absorption spectrum (Valeur 2002). Thus, in contrast to reflectance-based stimuli, fluorescence-based visual stimuli provide the same spectral response regardless of illumination conditions. Therefore, in the outdoor conditions that change during the day and from day to day, $\boldsymbol{p}$ of a fluorescent object is considerably less variable than that of the non-fluorescent reflective object. Another advantage of fluorescence is that this mechanism converts energy from absorbed UV light into emission in the visible spectral range (e.g., in the blue), thus enhancing the overall emittance in the visible range beyond what purely reflectance-based emittance can achieve.

It has been previously established that naïve bumble bees show a particular preference for wavelengths between 400 and 420 nm and that this preference persists even after extensive training to other colors (Gumbert 2000). This is consistent with our observations, as our most attractive traps have emittance in that wavelength region, and the attraction persists in a variety of habitats (e.g., Figs. S1 and S2). However, further studies are needed to establish whether similarly strong wild bee attraction would be possible upon highly selective excitation of either UV or green receptor type (Lunau and Maier 1995; Giurfa et al. 1995), which has not been provided by our traps.

Conclusion

We demonstrated strong attraction by a wide variety of wild bees to traps that under solar illumination emit light exclusively in the blue spectral region; such emittance is facilitated by sunlight-induced fluorescence. Alignment of spectral characteristics of traps with results of field studies indicates that excitation of the blue receptor type, as exclusive as possible, is the critical factor for achieving strong attraction. Our most successful traps are characterized by strong spectral overlap between the trap emittance and the photosensitivity characteristic of the blue receptor type, with minimal possible overlap with photosensitivity characteristics of the other two receptor types; traps that have spectral overlap with the blue receptor characteristic but also emit in other spectral regions were much less attractive, or not at all. Our studies illustrate that sunlight-induced fluorescence emission can be an efficient way of promoting highly selective excitation of a single receptor type. An empirical model that quantifies wild bee attraction to an object based on the optical properties of that object is proposed, which can be used as a guide in design of tools for manipulation and assessment of bee populations.

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

Ethical standard The ethical standards of the experiments comply with the current laws of the country in which they were performed.

Animal care Animal care standards comply with the current laws of the country in which they were performed. Reports of animal experiments must state that the "Principles of laboratory animal care" (NIH publication no. 85-23 revised 1985) were followed as well as specific national laws (e.g., the current version of the German Law on the Protection of Animals) where applicable.

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