Journal of Comparative Physiology A
https://doi.org/10.1007/s00359-018-1269-x

ORIGINAL PAPER

Understanding innate preferences of wild bee species: responses to wavelength-dependent selective excitation of blue and green photoreceptor types

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Received: 6 March 2018 / Revised: 27 May 2018 / Accepted: 29 May 2018

Abstract

Bees have a trichromatic vision with ultraviolet, blue, and green photoreceptors in their compound eyes. While the three photoreceptor types comprise the 'color space' at the perceptual level, preferential excitation of one or two of the photoreceptor types has been shown to play an important role in innate color preferences of bumble bees. Bees have been shown to exhibit strong attraction to fluorescence emission exclusively in the blue spectral region. It is not known if emission exclusively in the green spectral region produces similar attraction. Here, we examined responses of wild bees to traps designed to selectively stimulate either the blue or the green photoreceptor using sunlight-induced fluorescence in the 420–480 or 510–540 nm region, respectively. Additionally, we probed how subtle changes in the spectral characteristics of the traps affect the bee captures once a highly selective excitation of the blue photoreceptor is achieved. It was established that selective excitation of the green photoreceptor type was not attractive, in contrast to that of the blue photoreceptor type. However, once a highly selective excitation of the blue photoreceptor type (at ~400–480 nm) was achieved, the wild bees favored strong excitation at 430–480 nm over that in the 400–420 nm region.

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

Abbreviations

BF	Blue fluorescent
CN	Clear non-fluorescent
CBF	Clear blue fluorescent
GF	Green fluorescent
GN	Green non-fluorescent
IF	Purple trap I fluorescent
IN	Purple trap I non-fluorescent
JF	Blue trap J fluorescent
JN	Blue trap J non-fluorescent
KF	Blue trap K fluorescent
KN	Blue trap K non-fluorescent
UV	Ultraviolet

Introduction

Visual signals, such as the color of flowers, are used by a variety of pollinators including hummingbirds, hoverflies, butterflies, and bees for the detection of food resources (Rohde et al. 2013). For example, bees are able to utilize such signals for the detection of multiple food resources in landscapes that vary temporally and spatially in cues depending on the plant and/or flower species and the backgrounds against which they are perceived. Many aspects of bee color vision have been investigated, and well-developed color space models provide support for experimental data on bee attraction to flowers of different colors and patterns (Spaethe et al. 2001; Lunau et al. 2006; Wertlen et al. 2008;

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00359-018-1269-x) contains supplementary material, which is available to authorized users.

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Published online: 05 June 2018

Morawetz and Spaethe 2012). Bees have a trichromatic visual system comprised of ultraviolet (UV), blue, and green photoreceptors, and color space models detail how a visual signal received by these photoreceptors is processed at a neural level by the bee's brain (Chittka 1996). Such color processing by bees and other pollinators has influenced spectral signal evolution of flowers relying on these pollinators. However, in addition to neural processing, there is another important factor driving the flower spectral evolution, namely the innate color preferences of certain pollinators (Lunau 1990; Gumbert 2000; Simonds and Plowright 2004; Morawetz et al. 2013; Goodale et al. 2014; Dyer et al. 2016).

Studies of innate color preferences of bees have assessed the importance of spectral purity, color contrast, and dominant wavelength on the rates with which the naïve bee would choose a particular target (Lunau 1990). Several studies revealed that higher spectral purity targets and visual stimuli in the 400–420 nm (human violet) and, possibly, 510–520 nm (human green) wavelength ranges were preferred by naïve honey bees or bumble bees over signals of lower spectral purity and other wavelengths (Gumbert 2000; Lunau 1990). On the other hand, naïve stingless bees *Tetragonula carbonaria* Smith (previously known as *Trigona carbonaria*) preferred dominant wavelengths in the 420–460 nm (human blue) range, and spectral purity as a sole parameter did not correlate with the bee choices; instead, correlation between the bee choices and the green contrast of the target was observed (Dyer et al. 2016).

In our previous publication (Rao and Ostroverkhova 2015), we reported on extraordinary attraction across a wide variety of wild bee species (up to 92 species in 27 genera in 5 families in Western USA, 31 genera in 5 families elsewhere in the USA, and 48 species in 13 genera in 3 families in Australia), and in a diversity of landscapes, to a particular blue trap that exhibits strong fluorescence in the 420–480 nm spectral region under sunlight UV excitation. Under the same conditions, non-fluorescent blue traps, as well as fluorescent traps that emitted light across other wavelength regions, were not attractive. By analyzing spectral attributes of a variety of fluorescent and non-fluorescent traps and relating them to the number of bees attracted to each trap, we correlated the observed behavior with a range of parameters typically used in characterizing bee vision including green contrast and spectral purity. However, the best predictor of the trap attractiveness to the wild bees was determined to be a parameter, which we named the relative blue receptor contrast p_{blue}, that quantifies the degree to which the blue photoreceptor excitation is *exclusive*. The highest theoretically achievable value of p_{blue} is 1, at which only the blue photoreceptor type, and not the UV or green photoreceptor types of the bee, is excited. Because of the spectral overlap of photosensitivity characteristics of the three photoreceptor types, $p_{blue} = 1$ is unattainable and thus $p_{blue} < 1$. The minimal value of p_{blue} that was necessary for a trap to be significantly attractive to wild bees varied across different backgrounds and bee species. For example, for a green vegetation background, $p_{blue} > 0.6$ was needed for a trap to be attractive to the bumble bees, and such high values could only be achieved with traps strongly fluorescent in the relevant wavelength range. In this paper, we build on our previous work by examining, under similar outdoor conditions, (1) whether nearly exclusive excitation of the *green* photoreceptor type is attractive to the wild bees and (2) once a nearly exclusive excitation of the *blue* photoreceptor type is achieved, how the degree of attraction depends on the exact spectral characteristics of the trap.

Materials and methods

The responses of wild bees to traps with different spectral characteristics were examined under outdoor conditions. 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 (Stephen and Rao 2005; Hudon and Plowright 2011; Rao and Ostroverkhova 2015). No odor was added to the traps.

Table 1 summarizes properties of the traps used in our studies. Clear non-fluorescent cross vanes (Fig. 1) and screw cap funnels (CN) served as the controls. In all traps, clear non-fluorescent screw cap funnels were used. CN and BF vanes, both of which were used in our previous studies (Rao and Ostroverkhova 2015), were obtained from Spring Star (Woodinville, WA, USA). For the traps GN, JN, JF, KN, KF, IN, and IF we wrapped the clear vanes (CN) with Roscolux sheet color filters obtained from Stage Lighting Store, Inc., with product numbers (e.g. R68S) and the manufacturer color designation (e.g. "Parry Sky Blue") shown in Table 1. The CBF, JF, KF, and IF vanes were obtained by painting the CN, JN, KN, and IN vanes, respectively, with a clear blue fluorescent paint (paint 1 in Table 1) from Risk Reactor, Inc. The paint appears clear under light with weak UV component but emits bright fluorescence in the blue wavelength region under UV excitation. To obtain the GF vanes, we painted the clear vanes CN with a Rosco green fluorescent paint (paint 2 in Table 1) obtained from Super F Paint, Inc. Appearances of selected vanes under ambient light with weak UV component are presented in Fig. 1.

Measurements of optical properties of traps and spectral data analysis

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

Table 1 Color (as perceived by a human observer under ambient light with weak UV component), fluorescence emission of traps (under UV excitation) used in field studies, and wavelength of peak fluorescence emission obtained under UV excitation

Trap	Wrap/paint	Color under ambient light	Fluorescence wavelength at max
BF (blue fluorescent)	None	Blue	430 nm
CN (clear non-fluorescent)	None	Clear	None
CBF (clear blue fluorescent)	CN + paint 1[a]	Clear	435 nm
GF (green fluorescent)	CN + paint 2[a]	Green	512 nm
GN (green non-fluorescent)	CN + R389S[b]	Green ("Chroma Key Green")[c]	None
JF (blue fluorescent)	CN + R68S[b] + paint 1[a]	Blue ("Parry Sky Blue")[c]	435 nm
JN (blue non-fluorescent)	CN + R68S[b]	Blue ("Parry Sky Blue")[c]	None
KF (blue fluorescent)	CN + R382S[b] + paint 1[a]	Blue ("Congo Blue")[c]	435 nm
KN (blue non-fluorescent)	CN + R382S[b]	Blue ("Congo Blue")[c]	None
IF (purple fluorescent)	CN + R349S[b] + paint 1[a]	Purple ("Fuchsia")[c]	435 nm
IN (purple non-fluorescent)	CN + R349S[b]	Purple ("Fuchsia")[c]	None

[a]Paint 1 = clear blue fluorescent paint, paint 2 = green fluorescent paint

[b]Rosco filter numbers as designated by the manufacturer (Roscolux)

[c]Color names of the Rosco filters as designated by the manufacturer

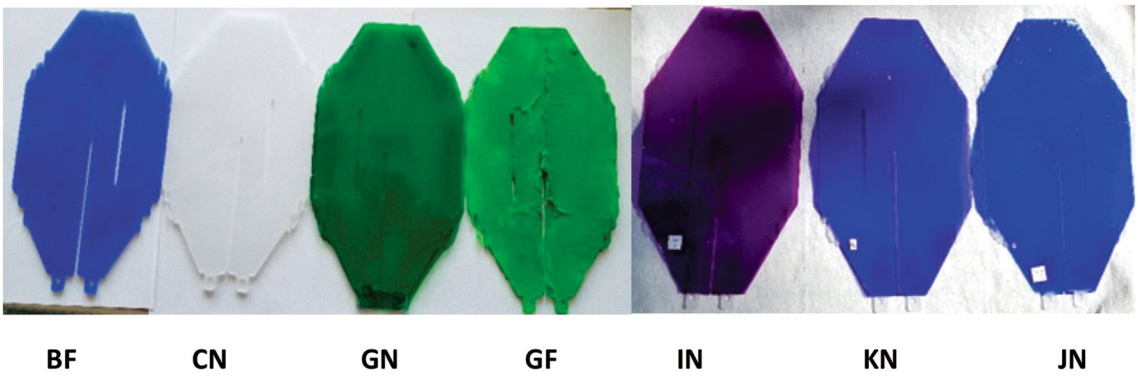

Fig. 1 Appearance of BF, CN, GN, GF, IN, KN, and JN vanes under ambient light with weak UV component. Fluorescent vanes CBF, IF, KF, and JF (obtained by painting CN, IN, KN, and JN vanes, respectively, with a clear paint that fluoresces under UV light) appear similar to CN, IN, KN, and JN, respectively, under ambient light with weak UV component and are not included in the figure

(solar simulator Oriel 96000 with AM1.5G filter), and measuring a signal emitted from the vane's front surface with a calibrated fiber-coupled spectrometer (Ocean Optics USB2000) as described in our previous publication (Rao and Ostroverkhova 2015). (Here the "AM" stands for "Air Mass" defined as $AM = 1/\cos(\theta)$, 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 reflect average solar illumination conditions in the USA.) Fluorescence in the vanes (Fig. 2) was also measured separately by exciting a vane with the 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 detecting an emitted signal in the same geometry as the total spectral emittance.

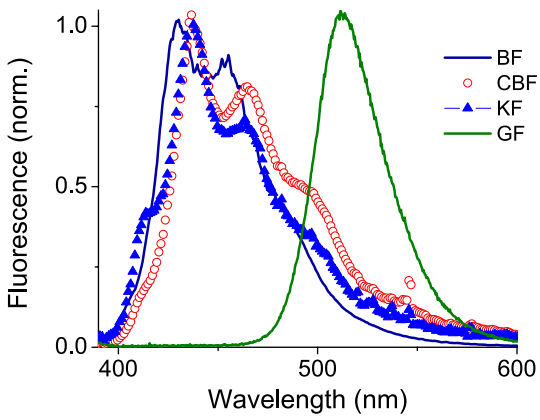

Fig. 2 Fluorescence from BF, CBF, KF, and GF vanes under UV excitation. Fluorescence spectra of JF and IF vanes are not included in the figure as they are identical to those of KF

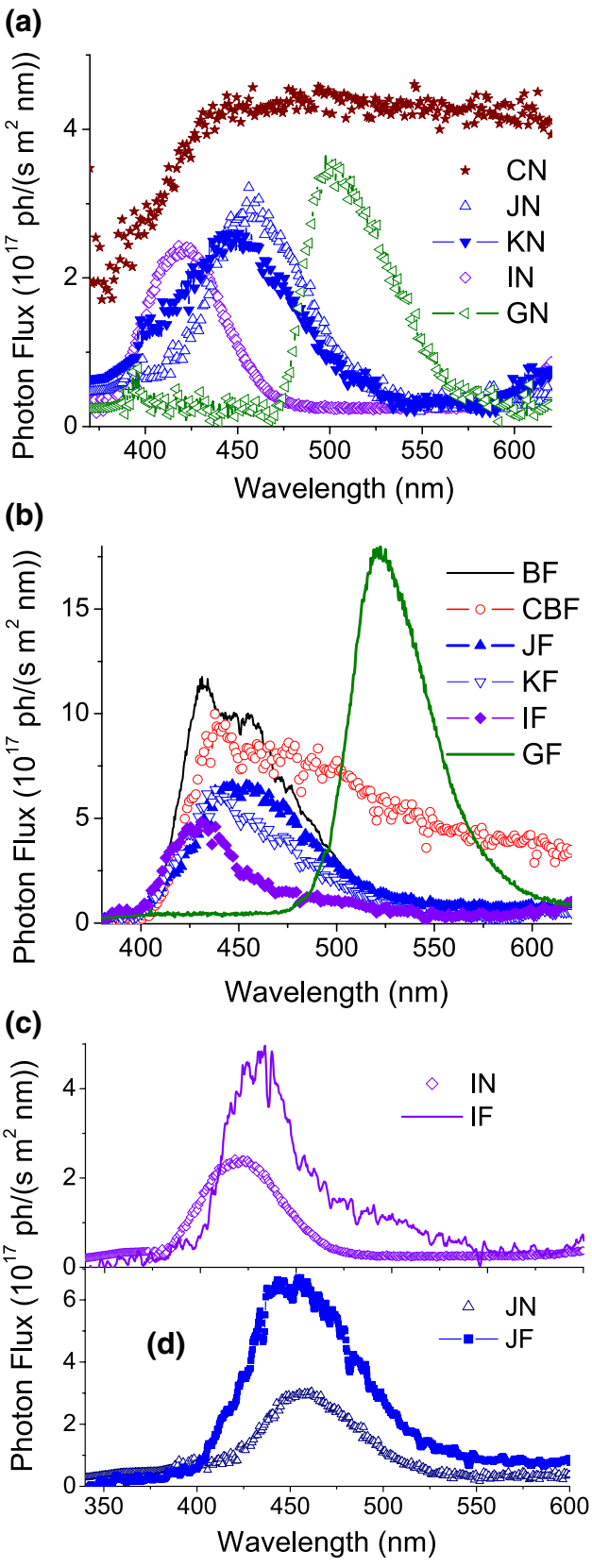

Fig. 3 Total photon flux emitted from **a** non-fluorescent vanes CN, JN, KN, IN, and GN and **b** fluorescent vanes BF, CBF, JF, KF, IF, and GF under solar AM1.5G illumination. **c, d** total emitted flux from IF vane (**c**) and JF vane (**d**) as compared to IN and JN, respectively, illustrating the effect of the fluorescent paint

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 photoreceptor type (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 photoreceptor type (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 a comparative analytical standard, those of the European bees *Bombus terrestris* (L.) (Apidae) (Peitsch et al. 1992). 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 photoreceptor type 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 (Rao and Ostroverkhova 2015). The parameters P_i were calculated for a green vegetation background (Peitsch et al. 1992). Based on the definition of P_i, for any given adaptation background $P_i = 1$.

The relative blue receptor contrast p_{blue} that quantifies the degree to which the blue photoreceptor excitation is *exclusive* was calculated as (Rao and Ostroverkhova 2015)

$$p_{\text{blue}} = P_{\text{B}} / \left(P_{\text{B}} + P_{\text{G}} + P_{\text{UV}} \right), \tag{2}$$

with the definition of Eq. (2), the highest theoretically achievable value of p_{blue} is 1, at which only the blue photoreceptor type, and not the UV or green photoreceptor types of the bee, is excited. However, because of the spectral overlap of photosensitivity characteristics of the three photoreceptor types, $p_{\text{blue}} = 1$ is unattainable and thus $p_{\text{blue}} < 1$. For the adaptation background ($P_i = 1$), $p_{\text{blue}} = 0.33$.

Field studies

The studies were conducted in an area dominated with agricultural crops foraged upon by wild bees for nectar and pollen. For each study, the traps were set up along the margins of private agricultural fields after permission was sought from the owner. None of the wild bees trapped were endangered or protected, and no specific permissions were required. At each site, the traps were separated by at least 7 m. The traps were set up for 2-day (Field Study 1 and 3) or 1-day (Field Study 2 when bee captures were high) periods, and bees that were collected were preserved and subsequently identified. The experiment was repeated over consecutive days (Field Study 1: 3 consecutive 2 day periods; Field Study 2: 3 consecutive 1 day periods; Field Study 3: 4 consecutive 2 day periods), and the traps were re-randomized with each set up.

Field Study 1 Wild bee response to the sunlight-induced fluorescence as a source of nearly exclusive excitation of the *blue* vs the *green* photoreceptor type.

To compare the impact of nearly exclusive excitation of the *blue* vs *green* photoreceptor type on bee captures, we compared wild bee captures in fluorescent blue (BF), fluorescent green (GF), non-fluorescent green (GN), and control (CN) traps. The BF traps exhibit strong fluorescence emission in the blue spectral region, peaked at ~430 nm (Fig. 2), which excited the *blue* photoreceptor type with a high degree of exclusivity (Rao and Ostroverkhova 2015). The GN trap did not have any fluorescence, but reflected mostly at wavelengths of the green photoreceptor sensitivity (~480–560 nm) (Fig. 3a). The GF trap was strongly fluorescent in the 500–570 nm spectral region (Fig. 2), exciting the *green* photoreceptor type almost as exclusively as possible (Fig. 3b).

Field Study 2 Wild bee response to *non-fluorescent* traps with reflection characteristics within the *blue* photoreceptor sensitivity.

Here, we compared bee captures in non-fluorescent human-perceived blue (JN, KN) and human-perceived purple (IN) traps (Fig. 1) with those in the BF and CN traps that served as "attractive" and "non-attractive" controls, respectively. The emission from traps JN, KN, and IN upon sunlight excitation is solely due to their reflection properties, with the wavelength of the peak emitted photon flux varying from ~420 nm for IN to ~450 nm for KN and ~460 nm for JN traps (Fig. 3a); all of these peak wavelengths are within the blue photoreceptor sensitivity (Fig. 4).

Field Study 3 Wild bee response to *fluorescent* and *non-fluorescent* traps with varied reflection spectra within the *blue* photoreceptor sensitivity.

In this study, we compared bee captures in fluorescent traps (JF, KF, IF, CBF) with those in their respective non-fluorescent counterparts (IN, KN, IN, CN) and with "attractive" and "non-attractive" control traps (BF and CN, respectively). Addition of a clear fluorescent paint to IN, KN, IN, and CN vanes reduced emittance in the UV spectral range (<400 nm), due to efficient absorption of UV light by the fluorescent dye, and considerably enhanced emittance in the blue spectral range (430–490 nm) corresponding to the fluorescence spectrum of the dye, as exemplified in Fig. 3c, d for the cases of IN vs IF and JN vs JF vanes, respectively. More details on changes of color in the bee color space in the fluorescent vs non-fluorescent vanes used in our studies can be found in the Supplementary Information and in Fig. S1.

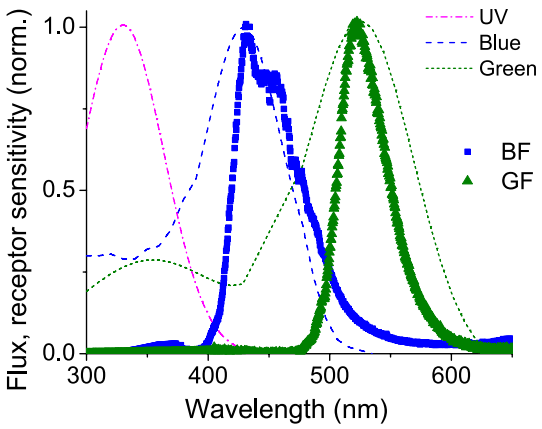

Fig. 4 Total emitted photon flux from fluorescent BF and GF vanes. Spectral sensitivity characteristics of UV, blue, and green photoreceptors are also shown. All data were normalized by their peak values

Data analysis

In Field Study 1, for 3 of the 4 treatments, no bees were captured during all days of the study and hence no statistical analysis was conducted. For Field Studies 2 and 3, bee catches in the different trap types were evaluated with a generalized linear model (proc GENMOD, SAS 9.4), using likelihood ratios (Type 3 analysis). The data from two trials were modeled using the negative binomial distribution, which had Goodness of Fit criteria of Deviance/DF of 1.1585, and 1.1627, respectively. Prior to the analysis of Field Study 2 data, 1.0 was added to all bee counts to adjust for the zero counts in one trap type. In Field Study 3, the four separate days of traps counts were treated as a Block factor to account for differences in weather that may have affected counts. Least square means comparisons used the Tukey–Kramer adjustment to account for multiple comparisons.

Results

Field Study 1 Wild bee response to the sunlight-induced fluorescence as a source of nearly exclusive excitation of the *blue* vs the *green* photoreceptor type.

The average numbers of wild bees captured in the traps are presented in Fig. 5. No bees were captured in traps GN, GF and CN during the entire duration of the study while an average of 11.9 bees per day per trap were captured in BF traps, hence no statistical analysis was conducted.

Field Study 2 Wild bee response to *non-fluorescent* traps with reflection characteristics within the *blue* photoreceptor sensitivity.

The average numbers of wild bees captured in the traps are presented in Fig. 6.

The generalized linear model using the negative binomial distribution indicated that the traps differed significantly in captures of wild bees ($\chi^2 = 31.47$, $df = 4$, $P < 0.0001$). Based on the Tukey–Kramer multiple comparison test, there were significantly more bees captured in the "attractive" control traps with blue fluorescent vanes (BF) compared with all other traps used in the study ($P < 0.05$). Traps with non-fluorescent blue vanes (JN and KN) significantly differed in bee captures from the "non-attractive" control trap CN ($P = 0.0018$ and 0.0022, respectively), but did not differ from each other ($P = 1.000$). The bee captures in the trap with purple non-fluorescent vanes IN did not differ significantly from the control trap CN ($P = 0.4001$), but differed from blue non-fluorescent traps JN and KN ($P = 0.0371$ and 0.0472, respectively). Similar trends were observed in bumble bees (*Bombus appositus Cresson, B. californicus Smith, B. griseocollis Degeer, B. mixtus Cresson, B. nevadensis Cresson, B. vosnesenskii Radoskowski*) which dominated the catches shown in Fig. 6, and in other wild bee species (Fig. S2).

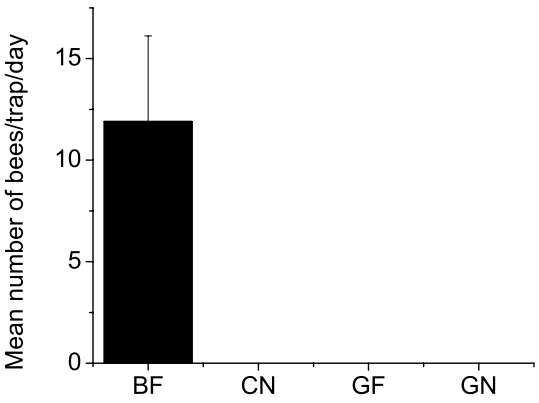

Fig. 5 The mean number of bee captures per trap per day (bars) in the first field study. Error bars represent standard error

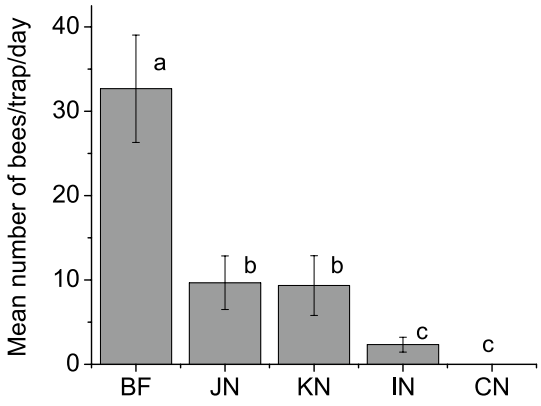

Fig. 6 The mean number of bee captures per trap per day (bars) in the second field study. Error bars represent standard error. Different letters above bars indicate statistically significant differences (Tukey's multiple comparison test)

Field Study 3 Wild bee response to *fluorescent* and *non-fluorescent* traps with varied reflection spectra within the *blue* photoreceptor sensitivity.

The average numbers of wild bees captured in the traps are presented in Fig. 7.

The generalized linear model using the negative binomial distribution indicated that the traps differed significantly in captures of wild bees ($\chi^2 = 108.68$, $df = 8$, $P < 0.0001$). The Tukey–Kramer pairwise comparison tests revealed that traps painted with a clear blue fluorescent paint exhibited significantly higher bee captures than their non-fluorescent counterparts in the case of traps CBF vs CN ($P < 0.0001$), JF vs JN ($P = 0.0357$) and KF vs KN ($P = 0.0007$), but not in the case of traps IF vs IN ($P = 0.9592$). Bee catches in the fluorescent traps JF and KF were not statistically different from those in the "attractive" control trap BF and from each other ($P > 0.05$). Interestingly, the IF traps (which appear purple to

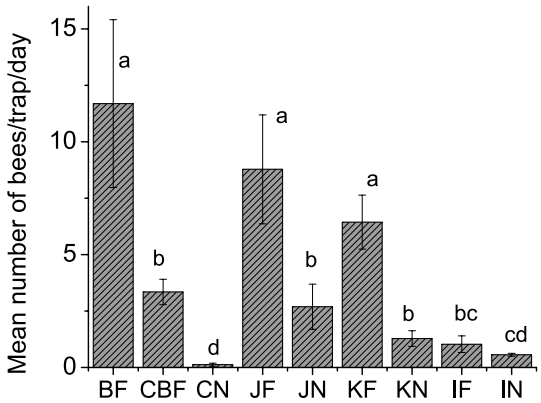

Fig. 7 The mean number of bee captures per trap per day (bars) in the third field study. Error bars represent standard error. Different letters above bars indicate statistically significant differences (Tukey's multiple comparison test)

a human eye under ambient light with weak UV component) consistently attracted significantly fewer bees than JF or KF traps (which appear blue to a human eye under the same light) ($P < 0.0001$), although the catches in the fluorescent purple traps IF were still significantly different from those in the "non-attractive" clear trap CN ($P = 0.0272$), similarly to significantly higher catches in the JF or KF traps with respect to CN ($P < 0.0001$).

Discussion

Selective excitation of one photoreceptor type: *blue* versus *green*

In our previous work (Rao and Ostroverkhova 2015), we identified a parameter p_{blue} of Eq. (2), which quantifies the degree to which the excitation of the blue photoreceptor is exclusive, to be well-correlated with the bee catches, across various adaptation backgrounds and wild bee species. The p_{blue} values of > 0.6 were needed to achieve significant attraction of the bumble bees when green vegetation is the adaptation background; the "attractive" control trap BF under these conditions has a high p_{blue} of 0.71. If a similar mechanism existed for the attraction caused by nearly exclusive excitation of the *green* photoreceptor type, then a relevant parameter would be

$$p_{\text{green}} = P_{\text{G}} / \left(P_{\text{B}} + P_{\text{G}} + P_{\text{UV}} \right), \tag{3}$$

where P_i's are the receptor-specific contrasts of Eq. (1). The p_{green} parameter is a measure of how exclusive the excitation of the green photoreceptor type is, and it is presented in Fig. 8 for the case of the BF, CN, GF, and GN traps used in Field Study 1. In spite of p_{green} reaching the value of 0.71 in the green fluorescent trap GF, no attraction of the wild bees to this trap or to a non-fluorescent green trap GN with $p_{\text{green}} = 0.4$ was observed (Fig. 5). This leads us to conclude that nearly exclusive excitation of the green photoreceptor type does not attract the wild bees, in contrast to the nearly exclusive excitation of the blue photoreceptor type. We note that we also attempted to probe whether nearly exclusive excitation of the UV photoreceptor type was attractive. However, the highest parameter p_{UV} (obtained by replacing P_{G} with P_{UV} in the numerator of Eq. (3)) provided by our traps was only 0.5. The number of bee captures in these traps were not significantly different from those in the "non-attractive" control trap CN. However, p_{UV} of at least 0.6–0.7 would be needed to reach a definitive conclusion regarding the presence or the absence of wild bee attraction to the exclusively UV-emitting traps.

Fluorescence as a facilitator of highly selective excitation of a single photoreceptor type

As discussed in detail in our previous publication (Rao and Ostroverkhova 2015), fluorescence may considerably enhance the degree to which the excitation of a single photoreceptor type is exclusive. Here, we focus on the blue photoreceptor type and the parameter p_{blue} that quantifies the degree of this exclusivity. Figure 9 shows p_{blue} values for various fluorescent and non-fluorescent traps used in our studies with respect to that of the "non-attractive" control trap CN (for which $p_{\text{blue}} = 0.35$). It is apparent that addition of a clear blue fluorescent paint to non-fluorescent CN, JN, KN, and IN vanes which yields fluorescent CBF, JF, KF, and IF vanes, respectively, considerably boosts values of p_{blue}. For example, fluorescent vanes JF, KF, and IF reach p_{blue} values of ~0.7, similar to that for the "attractive" control trap BF; these are higher by 0.2–0.25 than those for the corresponding non-fluorescent traps JN, KN, and IN.

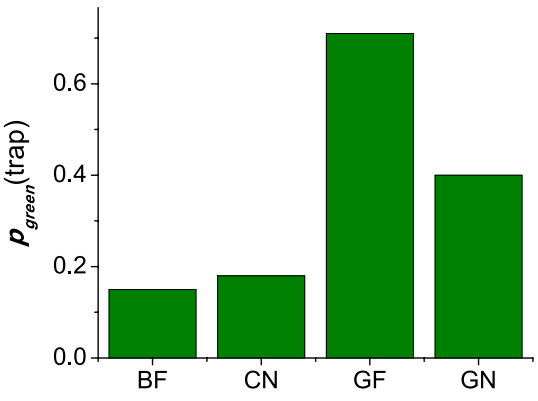

Fig. 8 Relative green receptor contrast p_{green} of Eq. (3) for BF, CN, GF, and GN vanes used in the first field study for the bumble bee, assuming green vegetation background

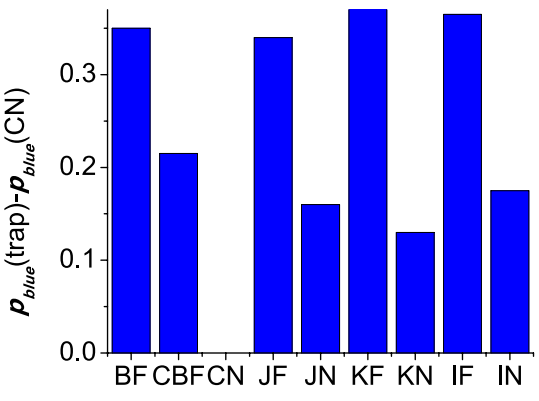

Fig. 9 The relative blue contrast parameter p_{blue} of Eq. (2) for each trap used in the third field study relative to that of the control trap CN for the bumble bee, assuming green vegetation background ($p_{\text{blue}}(\text{CN}) = 0.35$)

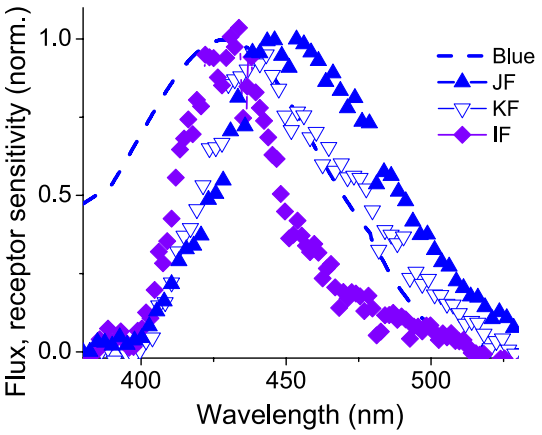

Fig. 10 Total emitted photon flux from fluorescent JF, KF, and IF vanes. Spectral sensitivity characteristic of the blue photoreceptor is also shown (dashed line). All data were normalized by their peak values

Therefore, the traps with JF, KF, and IF vanes would be expected to be considerably more attractive to the wild bees than those with JN, KN, and IN vanes. The trap CBF with the p_{blue} of ~0.58 would be expected to be somewhat less attractive (as this value is right around ~0.6 which serves as an approximate threshold for reliable attraction of bumble bees to these traps with a green vegetation background) (Rao and Ostroverkhova 2015). The non-fluorescent traps JN, KN, IN with the p_{blue} of 0.49–0.52 are borderline and would be expected to be considerably less attractive for the wild bees. Most of these expectations, based entirely on the p_{blue} value, correlate well with results from our field studies (Figs. 6, 7). However, there is an interesting exception, namely the IF traps which captured significantly fewer bees than the JF or KF traps in spite of the comparably high p_{blue} values of ~0.7 in all three cases. In particular, although the Field Study 3 did show that the bee catches in the IF traps were significantly different from those in the "non-attractive" control traps CN, the JF and KF traps were not only significantly different from CN, but also as attractive as the "attractive" control traps BF (Fig. 7).

The spectral comparison of the normalized photon fluxes emitted from the IF, JF, and KF vanes under sunlight illumination, superimposed with that of the blue photoreceptor sensitivity, is shown in Fig. 10. There are two clear differences in the spectra of the "attractive" JF and KF traps (which appear blue to the human eye) as compared to that of the "not-as-attractive" IF trap (which appears purple to the human eye): (1) less emphasized contribution of the 410–430 nm emission and (2) more emphasized emission in the 430–490 nm region in the "attractive" JF and KF traps. Therefore, it appears that reaching high p_{blue} values is a necessary, but not a sufficient condition for a strong attraction of the wild bees to sunlight-illuminated fluorescent traps.

Based on these observations, we hypothesize that the innate preference of wild bees tends toward a ~430–470 nm wavelength region rather than the 400–420 nm region identified in earlier studies of naïve bumble bees (Gumbert 2000). Our findings align well, for example, with recent results of a study of innate preferences of the Australian stingless bee *T. carbonaria* (Dyer et al. 2016). In that study, the preference peaks at about ~440 nm, and it drops off considerably faster in the 400–440 nm region than in the 440–500 nm region, consistent with our observations. Nevertheless, further studies are needed to quantitatively establish the additional criteria to be satisfied once the nearly exclusive excitation of the blue photoreceptor type has been reached, to achieve a strong attraction of wild bees to unrewarded outdoor sources of visual stimuli.

Conclusion

We demonstrated strong attraction of wild bees to fluorescent traps with emittance under solar illumination that nearly exclusively excited blue photoreceptor type. No such attraction was observed upon nearly exclusive excitation of the green photoreceptor type. Alignment of spectral characteristics of traps with results of field studies indicates that excitation of the blue photoreceptor type, as exclusive as possible, is the necessary, but not sufficient factor for achieving strong attraction. Our studies illustrate that the degree of wild bee attraction to targets emitting similar sunlight-induced fluorescence signal depends on the underlying reflection spectra of the targets within the spectral sensitivity of the blue receptor type. When nearly exclusive excitation of the blue photoreceptor type is achieved with trap emittances in the 400–490 nm region, the wild bees showed stronger preference for the traps with higher emittance at 430–490 nm, as compared to 400–430 nm. Targets with appropriately designed reflection and fluorescence spectra represent an efficient way of promoting highly selective excitation of a single photoreceptor type which is valuable for the design of tools for manipulation and assessment of wild bee populations.

Acknowledgements We thank the farmers for permitting the study on their farms. Funding from Agricultural Research Foundation and Oregon State University is acknowledged.

Compliance with ethical standards

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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