© 2020. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2020) 223, jeb220350. doi:10.1242/jeb.220350

RESEARCH ARTICLE

Polarized light sensitivity in *Pieris rapae* is dependent on both color and intensity

Adam J. Blake*, Gina S. Hahn, Hayley Grey, Shelby A. Kwok, Deby McIntosh and Gerhard Gries

ABSTRACT

There is an ever increasing number of arthropod taxa shown to have polarization sensitivity throughout their compound eyes. However, the downstream processing of polarized reflections from objects is not well understood. The small white butterfly, *Pieris rapae*, has been demonstrated to exploit foliar polarized reflections, specifically the degree of linear polarization (DoLP), to recognize host plants. The well-described visual system of *P. rapae* includes several photoreceptor types (red, green, blue) that are sensitive to polarized light. Yet, the roles and interaction among photoreceptors underlying the behavioral responses of *P. rapae* to stimuli with different DoLP remain unknown. To investigate potential neurological mechanisms, we designed several two-choice behavioral bioassays, displaying plant images on paired LCD monitors, which allowed for independent control of polarization, color and intensity. When we presented choices between stimuli that differed in either color or DoLP, both decreasing and increasing the intensity of the more attractive stimulus reduced the strength of preference. This result suggests that differences in color and DoLP are perceived in a similar manner. When we offered a DoLP choice between plant images manipulated to minimize the response of blue, red, or blue and red photoreceptors, *P. rapae* shifted its preference for DoLP, suggesting a role for all of these photoreceptors. Modeling of *P. rapae* photoreceptor responses to test stimuli suggests that differential DoLP is not perceived solely as a color difference. Our combined results suggest that *P. rapae* females process and interpret polarization reflections in a way different from that described for other polarization-sensitive taxa.

KEY WORDS: Butterfly, Insect vision, Polarization vision, Degree of linear polarization, Behavior

INTRODUCTION

Polarized light cues are used by many arthropods but apart from polarized skylight navigation little is known about how these organisms perceive polarized reflections (Heinloth et al., 2018). All organisms with rhabdomeric photoreceptors have the potential to sense polarized light (Horváth and Varjú, 2004). The tubular structure of the microvilli forming the rhabdom results in photopigments aligning more along the long axis of the microvilli. This alignment, in turn, causes these photopigments to be more sensitive to light vibrating in the plane parallel to the long axis of the microvilli (Johnsen, 2011). The plane of polarization with the greatest photoreceptor sensitivity is referred to as ϕ_{max} and typically aligns with the microvillar orientation (Horváth and Varjú, 2004). The size of this difference in sensitivity is referred to as polarization sensitivity (PS) and is defined as the the ratio of sensitivity to light vibrating at ϕ_{max}, and to light vibrating orthogonal to ϕ_{max}. Photoreceptors with a high PS are typically found in a specialized area of the compound eye known as the dorsal rim, allowing for polarized skylight navigation (Labhart and Meyer, 1999). The microvilli of these photoreceptors are aligned, and untwisted, along the length of their relatively short rhabdom, thereby enhancing PS. Additionally, these high PS photoreceptors involved in skylight navigation, which differ in ϕ_{max}, all share similar spectral sensitivities. If these photoreceptors differed in both spectral sensitivity and ϕ_{max}, the perceived color of an object would depend on both its reflection spectrum and its polarization (Wehner and Bernard, 1993). Many insects avoid polarization-induced false colors by twisting the direction of these microvilli along the length of the rhabdom, because otherwise the perceived color of objects would change as insects navigate through the environment. However, many other insects, especially those in aquatic and semi-aquatic habitats (Horváth and Csabai, 2014), possess photoreceptors with moderate PS throughout their compound eyes, and some of these insects do experience these polarization-induced false colors (Kelber et al., 2001). Histological and electrophysiological work has also revealed evidence for PS in many herbivorous insects (Ilić et al., 2016; Mishra, 2015; Wachmann, 1977).

Recently, small white butterfly (*Pieris rapae*) females have been shown to discriminate among potential host plants based on the polarization of light reflected from their foliage (Blake et al., 2019a). Like any shiny surface, the leaf surface preferentially reflects light oscillating parallel to that surface (Horváth et al., 2002; Shashar et al., 1998). This axis of polarization (AoP, 0–180 deg), as well as the degree to which the foliar reflection is polarized (degree of linear polarization, DoLP, 0–100%), are both strongly dependent upon the viewing angle and the location of the light source. However, AoP (unlike DoLP) is largely independent of leaf surface characteristics (Blake et al., 2019a). As only the specular component of the reflection is polarized, any leaf characteristics that affect the relative shininess or mattness also affect the DoLP. Decreasing the diffuse reflection through absorbance by pigments, scattering the specular reflection with epicuticular waxes or pigments, or undulations of the plane of the leaf's surface can all affect the DoLP of foliar reflections (Grant et al., 1993). Being dependent on these leaf characteristics, foliar DoLP can convey information about the host plant not conveyed by its color or intensity. Female *P. rapae* are able to discern cabbage host plants and potato non-host plants based on the lower DoLP of cabbage leaf reflections (Blake et al., 2019a). In choice bioassays, which

Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, V5A 1S6, Canada.

*Author for correspondence (adam@ajblake.info)

A.J.B., 0000-0002-4143-6981; G.S.H., 0000-0003-1945-7935; H.G., 0000-0002-2959-7109; S.A.K., 0000-0002-5702-7648; D.M., 0000-0002-4346-2737; G.G., 0000-0003-3115-8989

Received 19 December 2019; Accepted 18 May 2020

Journal of Experimental Biology (2020) 223, jeb220350. doi:10.1242/jeb.220350

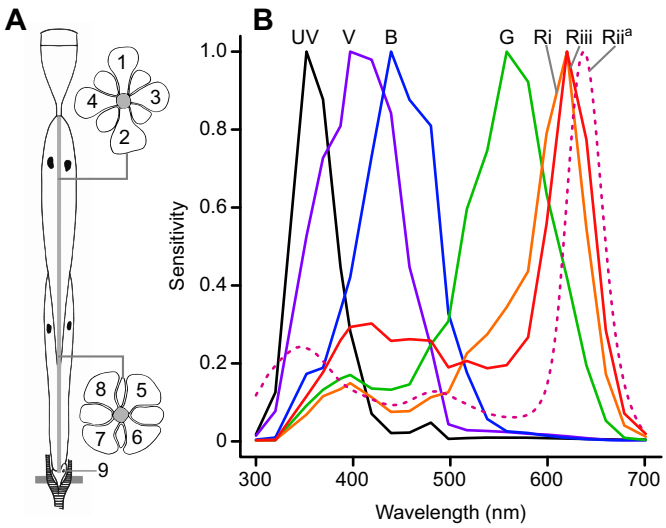

Fig. 1. Visual system of female *Pieris rapae*. (A) Diagram of ommatidium showing the arrangement of the nine photoreceptors (R1–9). (B) Spectral sensitivities, $S(\lambda)$, of the various photoreceptor spectral classes: ultraviolet (UV), violet (V), blue (B), green (G) and type I–III red (Ri–Riii). [a]Spectral sensitivity predicted from a model of the female ommatidium (Stavenga and Arikawa, 2011).

presented manipulated host plant images, *P. rapae* females rejected most images with a DoLP dissimilar to that of their cabbage host plant (DoLP of 31%). The informative value of this cue during host plant selection is enhanced by a relative insensitivity of *P. rapae* females to all but AoP very near to 45 or 135 deg. Both the underlying neurological mechanism and the photoreceptors involved in this discrimination remain unknown.

The visual system of *P. rapae* resembles that of other butterflies in that each ommatidium contains nine photoreceptors and the three ommatidial types are arranged in a random mosaic throughout the compound eye (Fig. 1A). Similar to the ommatidia of *Papilio* butterflies (Kelber, 2001), the shortwave-sensitive (UV, violet, blue) R1,2 photoreceptors, with the exception of the polarization-insensitive UV photoreceptor, respond most strongly to vertically polarized light, whereas the longwave-sensitive R3–9 photoreceptors respond most strongly to horizontally polarized light (R3,4) and obliquely polarized light (R5–8) (Blake et al., 2019b; Fig. 1B, Table 1). In the ventral portion of the eye, the sensitivity of the R5–8 photoreceptors, which, like R3,4, express a green-sensitive opsin, are modified by perirhabdomal filtering pigments into three classes of red photoreceptors distinct to the three ommatidial types, with more variation in PS among ommatidial types than reported in *Papilio* (Table 1). Of the shortwave receptors, only the type I blue photoreceptors show significant PS. There is also a lower PS in type II R3,4 receptors, whose polarization filtering effects on more basal photoreceptors (Snyder, 1973) may explain the difference in the axis of maximal polarization sensitivity (ϕ_{max}) of red photoreceptors among ommatidial types (Blake et al., 2019b). The R9 receptor is thought to be red-sensitive (Shimohigashi and Tominaga, 1991), and likely has low PS owing to its bidirectional microvillar arrangement (Qiu et al., 2002).

The compound eye of *P. rapae* has been extensively characterized, but there is no obvious mechanism that would explain how *P. rapae* processes the signals from its suite of photoreceptors to discriminate among stimuli with different DoLP. To determine whether *P. rapae* perceives differential DoLP as differences in stimulus intensity or color, we sought to emulate the work of Kinoshita et al. (2011). In two-choice bioassays, we examined the responses of *P. rapae* to differences in DoLP or color between stimuli to determine whether intensity differences between the stimuli affected preference in a similar manner. We also determined the photoreceptors involved in DoLP discrimination by minimizing the blue, red, or blue and red light of cabbage images that we presented to *P. rapae* in bioassays. This type of manipulation is possible through use of our novel monitor bioassay (Blake et al., 2019a). We predicted that if a photoreceptor were involved in DoLP discrimination, then image manipulations of stimuli reducing the photoreceptor's stimulation should alter the behavioral response of *P. rapae* to DoLP differences. We also modeled the catch of all *P. rapae* photoreceptors aiming to explain the observed behavioral bioassay responses of *P. rapae*.

MATERIALS AND METHODS

Insect material

Our laboratory colony of *P. rapae rapae* (Linnaeus 1758) originated from eggs obtained from the Carolina Biological Supply Company (no. 144100, Burlington, NC, USA) and later from adults collected from cabbage fields near Delta, BC, Canada. Using a well-established protocol (Webb and Shelton, 1988), larvae were maintained either on a wheat-germ diet or on cabbage plants grown in a greenhouse. We housed both male and female adults in indoor cages (60×60×60 cm, BugDorm 2120, MegaView Science Co. Ltd, Taichung, Taiwan) kept at 18–25°C and a photoperiod of 16 h:8 h light:dark. The females we tested in experiments were randomly selected from cohorts of adults 3–14 days post eclosion and were assumed to be gravid. We tested females in multiple bioassays, each bioassay presenting a new pair of experimental plant images. These different bioassays were considered independent.

General experimental setup

We used the same experimental arena (31.6×76.5×32.1 cm) and LCD monitor setup as recently described (Blake et al., 2019a;

Table 1. Summary of the spectral class and polarization characteristics of photoreceptors R1–9 in ommatidial types I–III

	I			II			III		
Photoreceptor	$S(\lambda)$	PS	ϕ_{max} (deg)	$S(\lambda)$	PS	ϕ_{max} (deg)	$S(\lambda)$	PS	ϕ_{max} (deg)
R1	UV	1.1	n/a	V	1.2	7	UV	1.1	n/a
R2	B	2.9	6	V	1.2	7	UV	1.1	n/a
R3,4	G	1.9	95	G	1.3[a]	91[a]	G	1.9	95
R5,7	Ri	2.2	155	Rii	1.9[a]	131[a]	Riii	2.1	156
R6,8	Ri	2.2	34	Rii	1.9[a]	52[a]	Riii	2.1	33
R9	R?	?	?	R?	?	?	R?	?	?

$S(\lambda)$, spectral sensitivity (see Fig. 1B); PS, polarization sensitivity; ϕ_{max}, axis of maximal polarization sensitivity; UV, ultraviolet; V, violet; B, blue; G, green; Ri–Riii, type I–III red. UV and B photoreceptors are positioned opposite each other but are equally likely to be in the R1 or R2 position.
[a]Values inferred from electrophysiological recordings of male butterflies.

Journal of Experimental Biology (2020) 223, jeb220350. doi:10.1242/jeb.220350

Fig. 2A). The inner surface of the removable arena lid was lined with matt white banner paper (NCR Corp., Duluth, GA, USA). We left the two end sections of the arena facing the monitors (stimulus windows) unobstructed but lined all the other inner surfaces of the arena with a matt brown kraft paper (NCR Corp.). To prevent build-up of any olfactory cues in the arena, we replaced the paper lining the interior surfaces and cleaned exposed glass surfaces with hexane daily.

In all experiments, we displayed cabbage plant images, created through photo polarimetry, as detailed in a recent publication (Blake et al., 2019a). In summary, we photographed cabbage plants, corrected the image color balance using a reflectance standard (SRS-99-010, Labsphere, NH, USA), removed the image background, and then standardized the plant size in each image such that all plant images presented an equal number of pixels. The pixel values of these red/green/blue (RGB) images were then manipulated to create versions that differed in intensity or color (Table S1, Fig. S1D–F). These images were presented on paired liquid crystal display (LCD) monitors (1707FPt, Dell Inc., Round Rock, TX, USA) calibrated to minimize any differences between monitors in the displayed irradiance spectra of pixels with identical RGB values (Fig. S1C). These monitors lack UV irradiance but the absence of UV wavelengths did not affect DoLP-based host plant preferences (Blake et al., 2019a). As LCD monitors produce highly polarized light, we manipulated the AoP by rotating the display and counter-rotating the image. Using a λ/4 retarder film (no. 88-253, Edmund Optics, Barrington, NJ, USA), we were also able to manipulate the plant image DoLP by changing the alignment of the AoP of the display relative to the retarder film (Blake et al., 2019ac). Using LCD monitors also enabled us to readily manipulate the plant image's color and/or intensity.

The monitors were separated from the stimulus windows of the experimental arena by a stimulus chamber (31×31×47 cm) lined with the same kraft paper as the arena. This separation limited the range of viewing angles of the monitor from within the arena. In order to limit the visible portion of the LCD to that displaying the plant image, we placed a kraft paper plant mask over the display aperture in each stimulus chamber (Fig. 2B). The top of each stimulus chamber had a lighting aperture (27×26 cm) covered with the same white banner paper as the arena lid, thus affording similar illumination of the arena and the stimulus chambers. The arena and the chambers were lit by a fluorescent lamp (Fig. S2B; F32T8/SPX50/ECO GE, Boston, MA, USA) centered 15 cm above the arena.

Using a camera mounted at the top rear of each stimulus chamber, we monitored the response of *P. rapae* females introduced into the arena. We allowed each female up to 5 min to approach one of the stimulus windows and recorded this approach as a behavioral response to the associated plant image. We considered females making no response as non-responders. Image stimuli were alternated so they appeared equally often on both monitors/sides of the arena. To help minimize any time-of-day (Lazopulo et al., 2019), day/weather (Pellegrino et al., 2013; Roitberg et al., 1993) or cohort-of-butterflies effects, we ran bioassays in blocks that included all stimuli comparisons using butterflies from a single cohort. There were two exceptions to this blocking: (1) the color-removal experiments commenced comparing R+G+B and G bioassay treatments at AoP 90 deg and only later included the remaining AoP 90 deg treatments; and (2) the AoP 0 deg bioassays in the color-removal experiment were a follow-up to the AoP 90 deg experiments and did not proceed concurrently.

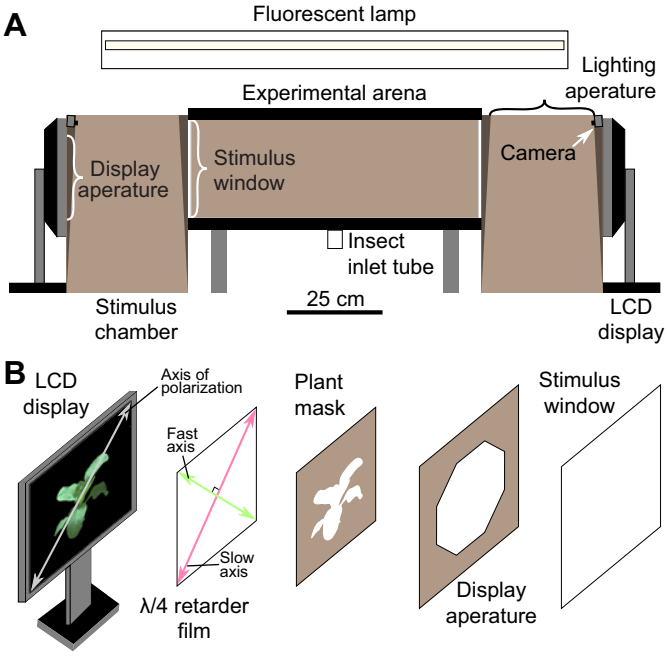

Fig. 2. LCD monitor bioassay setup. (A) Diagram of experimental arena. (B) Exploded view of components between the LCD monitors and the stimulus windows. After Blake et al. (2019a).

Intensity versus color discrimination experiment

To determine whether *P. rapae* females perceive differential DoLP as differential color or intensity, we performed experiments similar to those of Kinoshita et al. (2011). We presented females with paired stimuli consisting of the same cabbage image but modified to create differences in (A) intensity, (B) color and intensity or (C) DoLP and intensity between the two images (Fig. 3, Table S1). The paired stimuli we presented were (A) two unmodified images both with a DoLP of 31%; (B) one unmodified (treatment) image and one red-shifted (control) image each at a DoLP of 31% (Table S1, Fig. S1D, E); and (C) two unmodified images presented with a DoLP of either 31% (treatment) or 50% (control). The image whose intensity remained constant in each sub-experiment was designated the control, but this control image was not identical in each sub-experiment. In sub-experiments A–C, we presented the treatment image at intensities lower (44%, 87%), equal (100%) and greater (130%) than the original intensity (Table S1, Fig. S1D,E). In sub-experiment A, we did not present a choice between two unmodified images (DoLP 31%, 100% intensity), assuming no preference in response.

Color-removal experiment

To determine the photoreceptors involved in polarized light discrimination, we modified the color of cabbage images and offered *P. rapae* females a series of choices between these modified images presented at a DoLP of either 31% or 50%, with both images presented at an AoP of both 0 and 90 deg. To minimize the stimulation of the butterflies' red photoreceptors, blue photoreceptors or both simultaneously (within the limits inherent in the RGB color space where each color channel stimulates multiple photoreceptor classes; Fig. S1C), we set the red, blue, or red and blue values, respectively, of all pixels in both stimulus images to 0 (Table S1, Fig. S1F). As a control, we also offered a choice between images with no modification to any pixel values.

Journal of Experimental Biology (2020) 223, jeb220350. doi:10.1242/jeb.220350

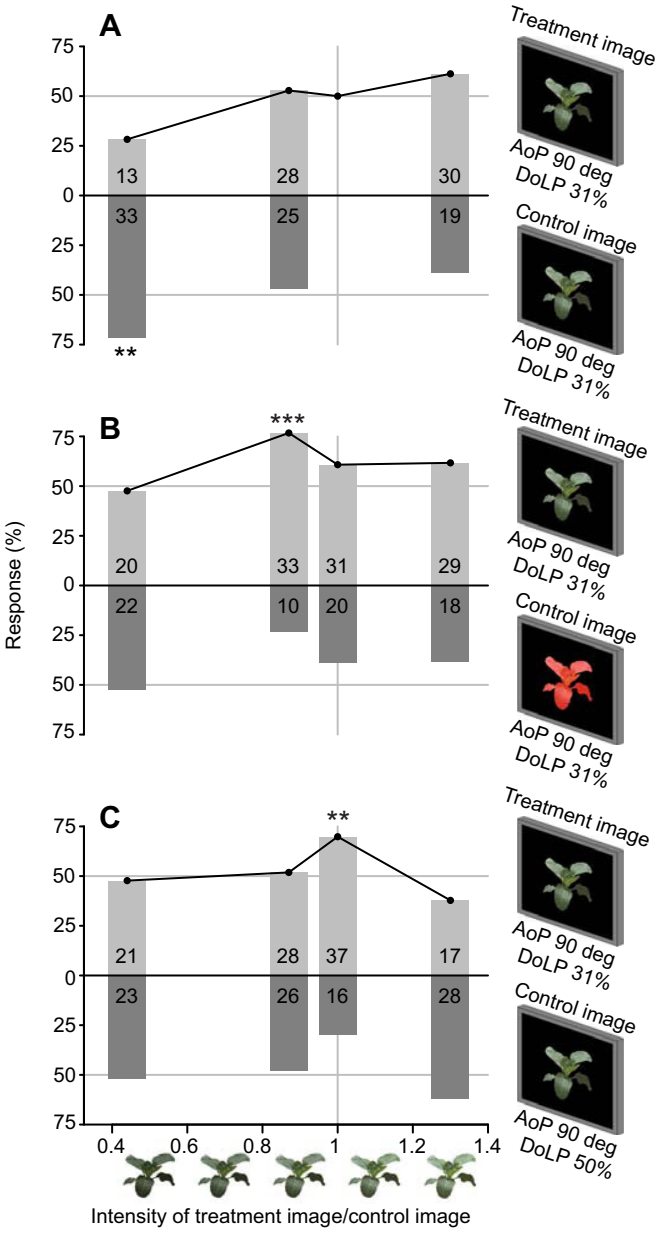

Fig. 3. Intensity versus color discrimination experiment. Effect of relative increase in intensity of the treatment image on the preference of *Pieris rapae* females when treatment and control images differ only in (A) intensity, (B) both color and intensity, and (C) both degree of linear polarization (DoLP) and intensity. The responses in A to treatment and control images of equal brightness were assumed to be 50%. Numbers of females responding to each stimulus are shown within bars. The asterisks indicate a significant proportion deviating from 50% (χ^2 test, $^{**}P<0.01$, $^{***}P<0.001$). AoP, axis of polarization.

Statistical analysis

We used two-tailed chi-square tests to determine whether the proportion of *P. rapae* females responding to plant images differed from 0.5, and whether the proportion of females responding differed among the experimental treatments. We excluded non-responding females from statistical analyses.

Modeling photoreceptor quantum catches

Unless otherwise noted, all spectra were measured with a calibrated spectrophotometer (HR-4000, Ocean Optics Inc., Dunedin, FL, USA) and were recently reported (Blake et al., 2020). To allow us to calculate the quantum catch of the background, we measured the ambient irradiance of the fluorescent lamps within the arena, the transmission of the arena wall and the λ/4 retarder film, and the reflectance of the brown kraft paper (Fig. S1A,B). We measured reflectance with a JAZ spectrometer (Ocean Optics) calibrated with a 99% Spectralon reflectance standard (SRS-99-010, Labsphere, NH, USA). Using photo polarimetry of the arena's interior (Foster et al., 2018), we approximated the mean DoLP and modal AoP of the background across the human visible spectrum (400–700 nm) to be 10% and 90 deg, respectively.

We also used this spectrometer to measure the irradiance produced by the monitors at a range of 8-bit RGB values including pure red, green and blue spectra ([255, 0, 0], [0, 255, 0], [0, 0, 255], respectively; Fig. S1C) in order to estimate the monitor's decoding gamma (γ=1.90) and the intensity at an RGB value of 0 (0.0055). Using Eqn 1, a modified gamma correction incorporating a non-zero intercept (Burger and Burge, 2009), we could appropriately scale and sum the pure spectra $I_C(\lambda)$ (where C is red, green or blue) using the red, green or blue pixel value PV_C to estimate the displayed irradiance spectra across all wavelengths (λ) from 300 to 750 nm for any combination of RGB values:

$$I(\lambda) = \sum_{C\in(\mathrm{R,G,B})} I_C(\lambda)\left(\frac{1}{1+0.0055}\right)\left(\frac{\mathrm{PV}_C}{255}\right)^{\gamma+0.0055}. \quad (1)$$

Using the mean RGB pixel values of the stimulus image, we could then create a mean spectrum for all pixels displayed in the image. The resulting spectrum was corrected for the transmission spectrum of the aquarium wall and the λ/4 retarder film (Fig. S1A).

Using wavelength-specific effects of the λ/4 retarder film (Blake et al. 2019c) along with measurements of a photoreceptor's PS, and the AoP of greatest sensitivity (ϕ_{max}) taken from Blake et al. (2019b), we used Eqn 2 to calculate the wavelength-specific effect of polarization on the photoreceptor's response [$P_i(\lambda)$ for photoreceptor type i]:

$$P_i(\lambda) = \frac{1}{\mathrm{PS}_i} + \frac{\mathrm{PS}_i - 1}{\mathrm{PS}_i} \cdot \left[\frac{1-\mathrm{DoLP}(\lambda)}{2} + \mathrm{DoLP}(\lambda)\cdot\cos^2(\mathrm{AoP}(\lambda)-\phi_{max})\right]. \quad (2)$$

This effect, along with the previously mentioned intensity spectrum, and the reported spectral sensitivities of *P. rapae* photoreceptors (R_i) (Blake et al., 2019b), allowed us to model the quantum catch (Q_i) of all photoreceptor types:

$$Q_i = \int_{300}^{750} I(\lambda)R_i(\lambda)P_i(\lambda)\mathrm{d}\lambda\,, \quad (3)$$

with dλ being the spectral resolution of the spectrometer used to measure $I(\lambda)$, and with all other variables interpolated to match this resolution (Blake et al., 2020). The quantum catch of the background (Q_{ib}) was similarly calculated, with irradiance $I(\lambda)$ being determined from the irradiance spectra of fluorescence lamps and the reflectance spectra of the kraft paper. However, the measured values of DoLP and AoP (10% and 90 deg, respectively) determined from photo polarimetry were assumed to be uniform

Journal of Experimental Biology (2020) 223, jeb220350. doi:10.1242/jeb.220350

across 300–750 nm. As photoreceptors adapt to the background illumination, we then calculated the quantum catch relative to the background (q_i):

$$q_i = Q_i/Q_{ib}. \quad (4)$$

RESULTS

Intensity versus color discrimination experiment

In general, when treatment and control stimuli differed only in intensity, *P. rapae* females preferred the more intense stimulus (Fig. 3A). This preference was statistically significant only when the intensity of treatment stimuli was <50% of that of the control stimuli (χ^2=8.70, N=1, P=0.0032). When the treatment stimulus had an intensity of 87% relative to the control stimulus, females did not discriminate between these stimuli.

When we presented a choice between a red-shifted cabbage image (control) and an unmodified (treatment) image of varying intensity, females significantly preferred the treatment image only with an intensity of 87% relative to the control image (χ^2=12.30, N=1, P=0.0005; Fig. 3B). Treatment images of a higher or a lower intensity were not significantly preferred, although there was a marginal preference for the treatment image when it had an intensity equal to, or greater than, that of the control image.

Similarly, when the treatment and control image differed in DoLP, females significantly preferred the treatment image (DoLP 31%) only when it had an intensity equal to that of the control image (DoLP 50%; χ^2=8.32, N=1, P=0.0039; Fig. 3C). Treatment images of a lower intensity were as attractive as the control image while there was a non-significant preference for the control image when it was more intense.

Color-removal experiment

When cabbage images were presented with all color channels intact (R+G+B), *P. rapae* females preferred the image with the lower DoLP both at an AoP of 0 and 90 deg (AoP of 0 deg: χ^2=7.36, N=44, P=0.0067; AoP of 90 deg: χ^2=15.25, N=63, P=0.0001; Fig. 4). When the blue color channel was removed (R+G), females shifted their preference towards the image with a higher DoLP, but only at an AoP of 0 deg (χ^2=18.75, N=86, P<0.0001). When the red color channel was removed (G+B), females preferred images with the higher DoLP at both AoPs (AoP of 0 deg: χ^2=11.72, N=53, P=0.0006; AoP of 90 deg: χ^2=7.41, N=39, P=0.0064). When only the green color channel of the image was included, females did not discriminate between images with a high or a low DoLP, when presented at an AoP of 90 deg. However, when these images were presented at an AoP of 0 deg, females chose the lower DoLP images (χ^2=9.28, N=57,

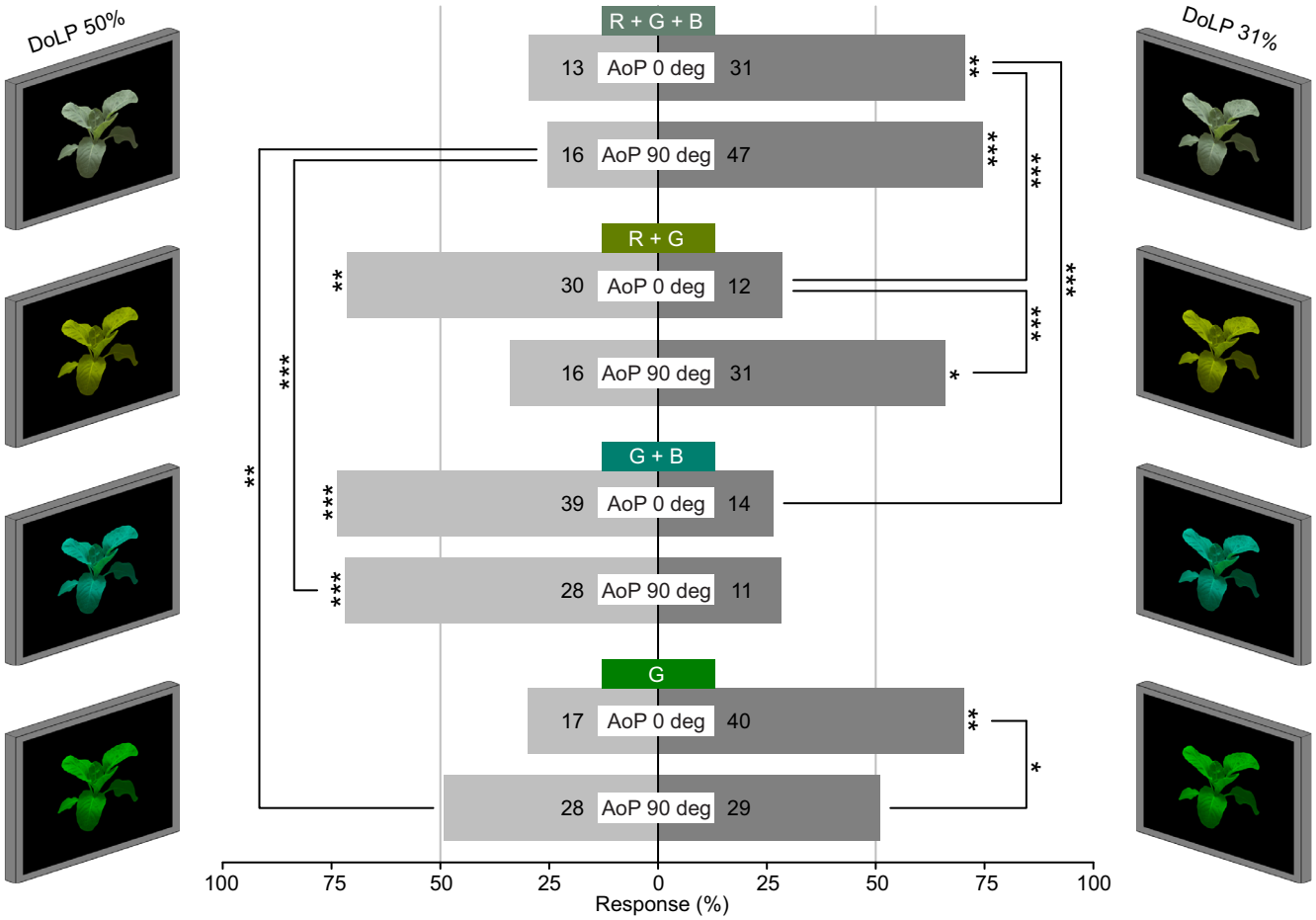

Fig. 4. Color-removal experiment. Changes in the preference of *P. rapae* females for cabbage plant images differing in degree of linear polarization (DoLP), with removal of one or more red (R), green (G), or blue (B) color channels. The stimulus images display unmodified RGB pixel values or have the red, blue, or red and blue values of all pixels in both stimulus images set to 0 (top to bottom). Numbers of females responding to each stimulus are shown within bars. The asterisks indicate a significant proportion deviating from 50% or a significant difference between two proportions (χ^2 test, *P<0.05, **P<0.01, ***P<0.001). Note: the 31% DoLP is typical of cabbage plants. AoP, axis of polarization.

Journal of Experimental Biology (2020) 223, jeb220350. doi:10.1242/jeb.220350

P=0.0023), similar to their response when all color channels were intact.

DISCUSSION

Our study refines the possible neurological processing mechanisms for DoLP-based host plant discrimination by female *P. rapae*. According to our data, *P. rapae* females are likely not perceiving differences in DoLP as differences purely in intensity or in color. Rather, our data suggest that perception of color, intensity and polarization, at least in the context of host plant discrimination, are all linked and contingent upon one another.

The intensity versus color discrimination experiment revealed that females preferred the plant image with greater intensity when all other factors were equal (Fig. 3). In our study, color preferences shifted in response to intensity changes in one of the two test stimuli, contrasting with results obtained in similar studies with *Papilio* butterflies (Kelber and Pfaff, 1999; Kinoshita et al., 2011). Although it is possible that *P. rapae* lacks true color vision (the ability to discriminate between colors independent of intensity), this explanation seems unlikely given the shared evolutionary history of *Papilio* and *Pieris* butterflies as members of Papilionoidea (Wahlberg et al., 2005), and the similarities of their respective compound eyes (Kelber et al., 2001). Although our colored stimuli lacked an appreciable UV component (unlike many stimuli tested with *Papilio*), these stimuli should provide adequate stimulation of the UV photoreceptors to distinguish between stimuli in the color-removal experiment (Fig. S2B). Training of bioassay insects offers a more likely explanation for these contrasting results. Although we tested the innate preferences of *P. rapae* females, corresponding studies with *Papilio* involved rewarded training (Kelber and Pfaff, 1999; Kinoshita et al., 2011). The spontaneous color choices of *Pieris brassicae* also shift in accordance with stimulus intensity (Scherer and Kolb, 1987); however, increases in intensity always have a positive effect on preference, contrasting our color preference data (Fig. 3B). When paired images were similar in color and DoLP, we observed a positive linear relationship between the intensity of the treatment image and the preference of female *P. rapae* for this image (Fig. 3A). In contrast, when image pairs were dissimilar in color or dissimilar in DoLP, female preference for the treatment image declined when the intensity of the treatment image was greater than that of the control image (Fig. 3B,C). Like in experiments with *Papilio*, these results suggest that *P. rapae* butterflies perceive differences in DoLP in a manner similar to their perception of differences in color, albeit not independent of intensity.

The color-removal experiment revealed that blue, green and red photoreceptors are involved in the perception of differential DoLP. This conclusion is based on data showing: (i) preferential responses to images with a lower DoLP (AoP: 0 and 90 deg) when all color channels were present; (ii) a preference shift for images (AoP: 0 or 90 deg) where either the blue or the red channel was removed; and (iii) the reversal of preferences with the green-only channel images (AoP: 90 deg) as compared with R+G or G+B images (AoP: 90 deg).

Contrary to results of the intensity versus color discrimination experiment, modeling of photoreceptor catch does not support the concept that differences in DoLP are perceived as color differences, at least not when modeled as a linear interaction among photoreceptors (Kelber, 1999, 2001). The color triangles represent the modeled *P. rapae* color space and depict the relative quantum catch of the red, green and shortwave (omitting UV in type I) photoreceptors of the three ommatidial types disregarding intensity

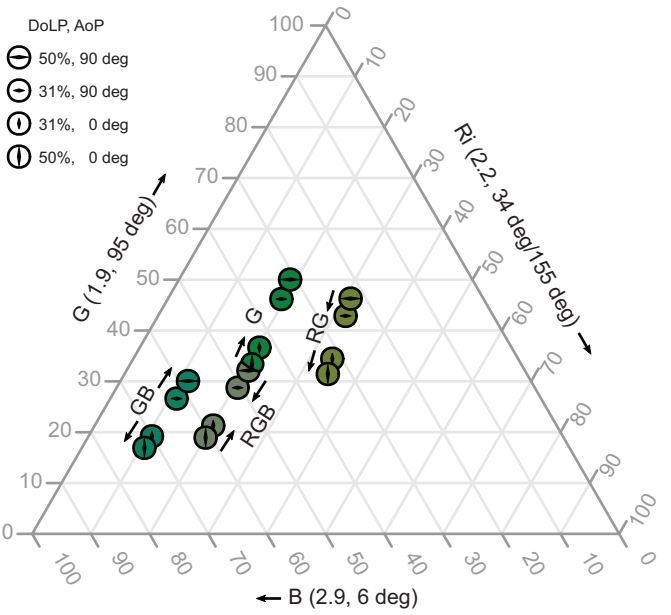

Fig. 5. Color triangle representing the modeled color space of *Pieris rapae* females. This triangle shows a model of relative blue (B), green (G) and red (Ri) photoreceptors' quantum catch in type I ommatidia. This color does not include the ultraviolet (UV) photoreceptor, which was deemed acceptable owing to the low levels of illumination in the UV range and the low PS of UV photoreceptors. The numbers in parentheses show the polarization sensitivity (PS) and axis of maximal polarization sensitivity (ϕ_{max}) of each receptor. The colored circles show the stimuli in the color-removal experiment. Arrows indicate the stimuli preferred by female *P. rapae*. DoLP, degree of linear polarization; AoP, axis of polarization.

(Fig. 5, Fig. S2). In modeling the catch of the red photoreceptors, we assumed the catch of R5–8 are pooled, negating much of the PS of these photoreceptors. If DoLP discrimination could be explained through linear interactions between different photoreceptors, as seen in *Papilio* and in *P. rapae* with unpolarized stimuli, we would expect a consistent direction of preference between stimuli. For example, using existing linear color models for *Papilio* and *Pieris*, with the catch of green photoreceptors having a positive effect and blue and red receptors having a negative effect, we would expect the stimuli closest to the upper green vertex to be preferred. In our modeling, stimuli differing only in polarization characteristics largely align along the blue to green axis, with the direction of preference among paired stimuli tested converging on no one region of the color space (Fig. 5). This inconsistency applied to all ommatidial types (Fig. S2), albeit with smaller separations among low and high DoLP stimuli owing to lower PS of the photoreceptors. It is unlikely that this inconsistency could be resolved even if downstream opponent processing was considered (Chen et al., 2019) or if photoreceptors were to be compared among different ommatidial types (Takemura and Arikawa, 2005).

Other plausible mechanisms also fail to explain our bioassay results. If polarization discrimination by *P. rapae* were to be dependent on comparisons between any two polarization-sensitive photoreceptors, or between one polarization-sensitive and one insensitive photoreceptor, we would expect AoP to have a strong effect on preference (Fig. S3A) (How and Marshall, 2014), similar to how *Papilio* butterflies strongly prefer horizontally over vertically polarized light (Kelber, 2001). We would also expect such comparisons among photoreceptors to result in either a linear increase or decrease in preferential response as DoLP increased

(Fig. S3B; How and Marshall, 2014). Yet, we found that the attractiveness of test stimuli was not affected by AoP outside regions near 45 and 135 deg, and that images with a DoLP similar to that of their cabbage host plants (DoLP of 31%) are preferred, with the appeal of stimuli declining both above and below this 31% value (Blake et al., 2019a). Comparisons between two or more pairs of photoreceptors are also unlikely to explain the observed DoLP preferences of *P. rapae* (Fig. S3E,F). Models that incorporated the absolute value of the differences in responses between photoreceptors (Fig. S3C,D) (Meglič et al., 2019) could explain observed AoP preferences in *P. rapae*, but again would fail to explain DoLP preferences. The results of the color-removal experiment preclude true polarization vision (the ability to discriminate among stimuli independent of color or intensity), as changes in color prompted large shifts in polarization preference.

Our combined results suggest a new and as of yet undescribed mechanism for the processing of polarized reflections underlying DoLP discrimination in *P. rapae*. The mechanism likely involves blue, green and red photoreceptor classes, and is affected by intensity, color and polarization. If true, this would be yet another example of unique neural processing of polarization information from object-reflected light. The systems for processing such information differ between all taxa thus far studied, including crabs (Smithers et al., 2019), fruit flies (Wernet et al., 2012), horse flies (Meglič et al., 2019), and backswimmers (Schwind, 1984). There are even as many as three different systems at work in *Papilio* butterflies depending on the behavioral context (Kelber et al., 2001; Kinoshita et al., 2011; Stewart et al., 2019). This information seems to show that different arthropod taxa have utilized the polarization sensitivity inherent in rhabdomeric photoreceptors to create visual subsystems tuned in accordance to their particular ecology. Further investigations into different arthropod taxa will almost certainly reveal novel combinations and processing of photoreceptor inputs using polarized light for object recognition.

Acknowledgements
We thank two anonymous reviewers for their helpful and constructive comments on this manuscript.

Competing interests
The NSERC-IRC to G.G. was supported by Scotts Canada Ltd as the industrial partner.

Author contributions
Conceptualization: A.J.B., G.G.; Methodology: A.J.B.; Software: A.J.B.; Formal analysis: A.J.B.; Investigation: G.S.H., H.G., S.A.K., D.M.; Data curation: A.J.B.; Writing - original draft: A.J.B.; Writing - review & editing: A.J.B., G.G.; Visualization: A.J.B.; Supervision: G.G.; Project administration: G.G.; Funding acquisition: G.G.

Funding
This study was supported by an Alexander Graham Bell Canadian Graduate Scholarship to A.J.B., Natural Sciences and Engineering Research Council of Canada (NSERC) Undergraduate Student Research Awards to G.S.H., H.G., S.A.K. and D.M., and by an NSERC-Industrial Research Chair (IRC) to G.G., with Scotts Canada Ltd as the industrial sponsor.

Data availability
Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.xgxd254bs (Blake et al., 2019c) and https://doi.org/10.5061/dryad.kd51c5b39 (Blake et al., 2020).

Supplementary information
Supplementary information available online at
https://jeb.biologists.org/lookup/doi/10.1242/jeb.220350.supplemental

References
Blake, A. J., Go, M. C., Hahn, G. S., Grey, H., Couture, S. and Gries, G. (2019a). Polarization of foliar reflectance: novel host plant cue for insect herbivores. *Proc. R. Soc. B Biol. Sci.* **286**, 20192198. doi:10.1098/rspb.2019.2198

Blake, A. J., Pirih, P., Qiu, X., Arikawa, K. and Gries, G. (2019b). Compound eyes of the small white butterfly *Pieris rapae* have three distinct classes of red photoreceptors. *J. Comp. Physiol. A* **205**, 553-565. doi:10.1007/s00359-019-01330-8

Blake, A. J., Go, M. C., Hahn, G. S., Grey, H., Couture, S. and Gries, G. (2019c), λ/4 retarder film measurement from: Polarization of foliar reflectance: novel host plant cue for insect herbivores. v7, Dryad, Dataset, https://doi.org/10.5061/dryad.xgxd254bs

Blake, A. J., Hahn, G. S., Grey, H., Kwok, S., McIntosh, D. and Gries, G. (2020). Spectral data and R code for modeling the photoreceptor catch of female *P. rapae* from: polarized light sensitivity in *Pieris rapae* is dependent on both color and intensity. Dryad, Dataset, https://doi.org/10.5061/dryad.kd51c5b39

Burger, W. and Burge, M. J. (2009). *Digital Image Processing: An Algorithmic Introduction Using Java*, 1st edn. New York: Springer.

Chen, P.-J., Belušič, G. and Arikawa, K. (2019). Chromatic information processing in the first optic ganglion of the butterfly *Papilio xuthus*. *J. Comp. Physiol. A* **206**, 199-216. doi:10.1007/s00359-019-01390-w

Foster, J. J., Temple, S. E., How, M. J., Daly, I. M., Sharkey, C. R., Wilby, D. and Roberts, N. W. (2018). Polarisation vision: overcoming challenges of working with a property of light we barely see. *Sci. Nat.* **105**, 27. doi:10.1007/s00114-018-1551-3

Grant, L., Daughtry, C. S. T. and Vanderbilt, V. C. (1993). Polarized and specular reflectance variation with leaf surface features. *Physiol. Plant.* **88**, 1-9. doi:10.1111/j.1399-3054.1993.tb01753.x

Heinloth, T., Uhlhorn, J. and Wernet, M. F. (2018). Insect responses to linearly polarized reflections: orphan behaviors without neural circuits. *Front. Cell. Neurosci.* **12**, 50. doi:10.3389/fncel.2018.00050

Horváth, G. and Csabai, Z. (2014). Polarization vision of aquatic insects. In *Polarized Light and Polarization Vision in Animal Sciences* (ed. G. Horváth), pp. 113-145. Berlin, Heidelberg: Springer.

Horváth, G. and Varjú, D. (2004). *Polarized Light in Animal Vision: Polarization Patterns in Nature*. New York: Springer.

Horváth, G., Gál, J., Labhart, T. and Wehner, R. (2002). Does reflection polarization by plants influence colour perception in insects? Polarimetric measurements applied to a polarization-sensitive model retina of *Papilio* butterflies. *J. Exp. Biol.* **205**, 3281-3298.

How, M. J. and Marshall, N. J. (2014). Polarization distance: a framework for modelling object detection by polarization vision systems. *Proc. R. Soc. B Biol. Sci.* **281**, 20131632. doi:10.1098/rspb.2013.1632

Ilić, M., Pirih, P. and Belušič, G. (2016). Four photoreceptor classes in the open rhabdom eye of the red palm weevil, *Rynchophorus ferrugineus* Olivier. *J. Comp. Physiol. A* **202**, 203-213. doi:10.1007/s00359-015-1065-9

Johnsen, S. (2011). *The Optics of Life*. Princeton, NJ: Princeton University Press.

Kelber, A. (1999). Ovipositing butterflies use a red receptor to see green. *J. Exp. Biol.* **202**, 2619-2630.

Kelber, A. (2001). Receptor based models for spontaneous colour choices in flies and butterflies. *Entomol. Exp. Appl.* **99**, 231-244. doi:10.1046/j.1570-7458.2001.00822.x

Kelber, A. and Pfaff, M. (1999). True colour vision in the orchard butterfly, *Papilio aegeus*. *Naturwissenschaften* **86**, 221-224. doi:10.1007/s001140050601

Kelber, A., Thunell, C. and Arikawa, K. (2001). Polarisation-dependent colour vision in *Papilio*. *J. Exp. Biol.* **204**, 2469-2480.

Kinoshita, M., Yamazato, K. and Arikawa, K. (2011). Polarization-based brightness discrimination in the foraging butterfly, *Papilio xuthus*. *Philos. Trans. R. Soc. B Biol. Sci.* **366**, 688-696. doi:10.1098/rstb.2010.0200

Labhart, T. and Meyer, E. P. (1999). Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microsc. Res. Tech.* **47**, 368-379. doi:10.1002/(SICI)1097-0029(19991215)47:6<368::AID-JEMT2>3.0.CO;2-Q

Lazopulo, S., Lazopulo, A., Baker, J. D. and Syed, S. (2019). Daytime colour preference in *Drosophila* depends on the circadian clock and TRP channels. *Nature* **574**, 108-111. doi:10.1038/s41586-019-1571-y

Meglič, A., Ilić, M., Pirih, P., Škorjanc, A., Wehling, M. F., Kreft, M. and Belušič, G. (2019). Horsefly object-directed polarotaxis is mediated by a stochastically distributed ommatidial subtype in the ventral retina. *Proc. Natl. Acad. Sci. USA* **116**, 21843-21853. doi:10.1073/pnas.1910807116

Mishra, M. (2015). An eye ultrastructure investigation of a plant pest *Acyrthosiphon pisum* (Harris) (Insecta: Hemiptera: Aphididae). *Open Access Insect Physiol.* **5**, 41-46. doi:10.2147/OAIP.S84633

Pellegrino, A. C., Peñaflor, M. F. G. V., Nardi, C., Bezner-Kerr, W., Guglielmo, C. G., Bento, J. M. S. and McNeil, J. N. (2013). Weather forecasting by insects: modified sexual behaviour in response to atmospheric pressure changes. *PLoS ONE* **8**, e75004. doi:10.1371/journal.pone.0075004

Qiu, X., Vanhoutte, K., Stavenga, D. G. and Arikawa, K. (2002). Ommatidial heterogeneity in the compound eye of the male small white butterfly, *Pieris rapae crucivora*. *Cell Tissue Res.* **307**, 371-379. doi:10.1007/s00441-002-0517-z

Roitberg, B. D., Sircom, J., Roitberg, C. A., van Alphen, J. J. M. and Mangel, M. (1993). Life expectancy and reproduction. *Nature* **364**, 108-108. doi:10.1038/364108a0

Scherer, C. and Kolb, G. (1987). Behavioral experiments on the visual processing of color stimuli in *Pieris brassicae* L. (Lepidoptera). *J. Comp. Physiol. A* **160**, 645-656. doi:10.1007/BF00611937

Schwind, R. (1984). Evidence for true polarization vision based on a two-channel analyzer system in the eye of the water bug, *Notonecta glauca*. *J. Comp. Physiol. A* **154**, 53-57. doi:10.1007/BF00605390

Shashar, N., Cronin, T. W., Wolff, L. B. and Condon, M. A. (1998). The polarization of light in a tropical rain forest. *Biotropica* **30**, 275-285. doi:10.1111/j.1744-7429.1998.tb00061.x

Shimohigashi, M. and Tominaga, Y. (1991). Identification of UV, green and red receptors, and their projection to lamina in the cabbage butterfly, *Pieris rapae*. *Cell Tissue Res.* **263**, 49-59. doi:10.1007/BF00318399

Smithers, S. P., Roberts, N. W. and How, M. J. (2019). Parallel processing of polarization and intensity information in fiddler crab vision. *Sci. Adv.* **5**, eaax3572. doi:10.1126/sciadv.aax3572

Snyder, A. W. (1973). Polarization sensitivity of individual retinula cells. *J. Comp. Physiol. A* **83**, 331-360. doi:10.1007/BF00696351

Stavenga, D. G. and Arikawa, K. (2011). Photoreceptor spectral sensitivities of the small white butterfly *Pieris rapae crucivora* interpreted with optical modeling. *J. Comp. Physiol. A* **197**, 373-385. doi:10.1007/s00359-010-0622-5

Stewart, F. J., Kinoshita, M. and Arikawa, K. (2019). Monopolatic motion vision in the butterfly *Papilio xuthus*. *J. Exp. Biol.* **222**, jeb191957. doi:10.1242/jeb.191957

Takemura, S.-Y. and Arikawa, K. (2005). Ommatidial type-specific interphotoreceptor connections in the lamina of the swallowtail butterfly, *Papilio xuthus*. *J. Comp. Neurol.* **494**, 663-672. doi:10.1002/cne.20830

Wachmann, E. (1977). Vergleichende Analyse der feinstrukturellen Organisation offener Rhabdome in den Augen der Cucujiformia (Insecta, Coleoptera), unter besonderer Berücksichtigung der Chrysomelidae. *Zoomorphologie* **88**, 95-131. doi:10.1007/BF01880649

Wahlberg, N., Braby, M. F., Brower, A. V. Z., de Jong, R., Lee, M.-M., Nylin, S., Pierce, N. E., Sperling, F. A. H., Vila, R., Warren, A. D. et al. (2005). Synergistic effects of combining morphological and molecular data in resolving the phylogeny of butterflies and skippers. *Proc. R. Soc. B Biol. Sci.* **272**, 1577-1586. doi:10.1098/rspb.2005.3124

Webb, S. and Shelton, A. M. (1988). Laboratory rearing of the imported cabbageworm. *New Yorks Food Life Sci. Bull.* **122**, 1-6.

Wehner, R. and Bernard, G. D. (1993). Photoreceptor twist: a solution to the false-color problem. *Proc. Natl. Acad. Sci. USA* **90**, 4132-4135. doi:10.1073/pnas.90.9.4132

Wernet, M. F., Velez, M. M., Clark, D. A., Baumann-Klausener, F., Brown, J. R., Klovstad, M., Labhart, T. and Clandinin, T. R. (2012). Genetic dissection reveals two separate retinal substrates for polarization vision in *Drosophila*. *Curr. Biol.* **22**, 12-20. doi:10.1016/j.cub.2011.11.028

Table S1. The mean RGB pixel values of plant images along with the corrections necessary to generate these images from the unmodified originals. The mean values were calculated from individual RGB means of each image. Also included are the degree of linear polarization (DoLP) and axis of polarization (AoP) of stimuli used in each experiment.

intensity-*vs*-color discrimination experiment (A) - intensity difference

treatment image						control image					
	R	G	B	DoLP	AoP		R	G	B	DoLP	AoP
	72 ± 1 (0.64×R)	82 ± 2 (0.64×G)	64 ± 2 (0.64×G)	31%	90°		112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	31%	90°
	105 ± 2 (0.93×R)	120 ± 3 (0.93×G)	93 ± 2 (0.93×G)	31%	90°		112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	31%	90°
	129 ± 3 (1.15×R)	148 ± 3 (1.15×G)	115 ± 3 (1.15×G)	31%	90°		112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	31%	90°

intensity-*vs*-color discrimination experiment (B) - color difference

treatment image						control image					
	R	G	B	DoLP	AoP		R	G	B	DoLP	AoP
	72 ± 1 (0.64×R)	82 ± 2 (0.64×G)	64 ± 2 (0.64×G)	31%	90°		237 ± 1 (0.38×R+195)	93 ± 2 (0.72×G)	82 ± 2 (0.82×G)	31%	90°
	105 ± 2 (0.93×R)	120 ± 3 (0.93×G)	93 ± 2 (0.93×G)	31%	90°		237 ± 1 (0.38×R+195)	93 ± 2 (0.72×G)	82 ± 2 (0.82×G)	31%	90°
	112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	31%	90°		237 ± 1 (0.38×R+195)	93 ± 2 (0.72×G)	82 ± 2 (0.82×G)	31%	90°
	129 ± 3 (1.15×R)	148 ± 3 (1.15×G)	115 ± 3 (1.15×G)	31%	90°		237 ± 1 (0.38×R+195)	93 ± 2 (0.72×G)	82 ± 2 (0.82×G)	31%	90°

intensity-*vs*-color discrimination experiment (C) - *DoLP* difference

treatment image						control image					
	R	G	B	DoLP	AoP		R	G	B	DoLP	AoP
	72 ± 1 (0.64×R)	82 ± 2 (0.64×G)	64 ± 2 (0.64×G)	31%	90°		112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	50%	90°
	105 ± 2 (0.93×R)	120 ± 3 (0.93×G)	93 ± 2 (0.93×G)	31%	90°		112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	50%	90°
	112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	31%	90°		112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	50%	90°
	129 ± 3 (1.15×R)	148 ± 3 (1.15×G)	115 ± 3 (1.15×G)	31%	90°		112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	50%	90°

color-removal experiment

	R	G	B	DoLP	AoP		R	G	B	DoLP	AoP
	112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	50%	90°		112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	31%	90°
	112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	0 ± 0 (0.00×G)	50%	90°		112 ± 2 (1.00×R)	129 ± 3 (1.00×G)	0 ± 0 (0.00×G)	31%	90°
	0 ± 0 (0.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	50%	90°		0 ± 0 (0.00×R)	129 ± 3 (1.00×G)	100 ± 3 (1.00×G)	31%	90°
	0 ± 0 (0.00×R)	129 ± 3 (1.00×G)	0 ± 0 (0.00×G)	50%	90°		0 ± 0 (0.00×R)	129 ± 3 (1.00×G)	0 ± 0 (0.00×G)	31%	90°

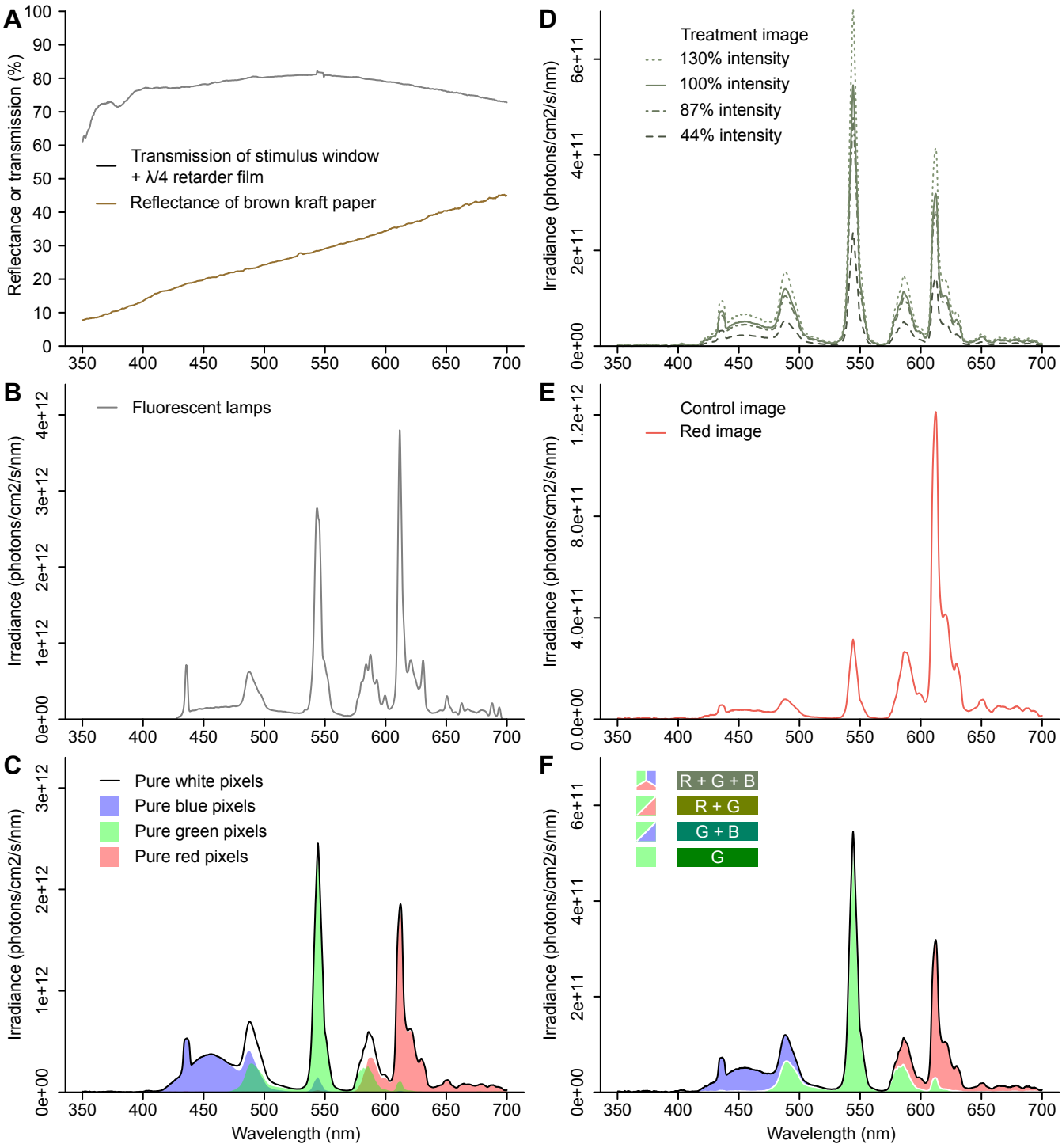

Fig. S1 Spectra of filters, background, and illumination sources. (A) Transmission spectrum of the stimulus windows of the experimental arena (Fig. 2) with a λ/4 retarder film and the reflectance spectrum of the background brown kraft paper. (B) Irradiance of the fluorescent lamps measured from within the arena at its center. (C) Irradiance of white (RGB: 255, 255, 255), blue (0, 0, 255), green (0, 255, 0), or red pixels (0, 0, 255) as measured from the other surface of the display of the bioassay monitors (mean of both LCD monitors). (D) Differences in irradiance spectra among different control image intensities used in the intensity-*vs*-color discrimination experiment. (E) Spectra of the red control image in the color difference portion of the intensity-*vs*-color discrimination experiment. (F) Spectra of stimuli tested in the color-removal experiment, where the red, blue, or red and blue, pixel values were set to 0. The spectra in D-F were calculated using equation 1 from the mean pixel values in Table S1.

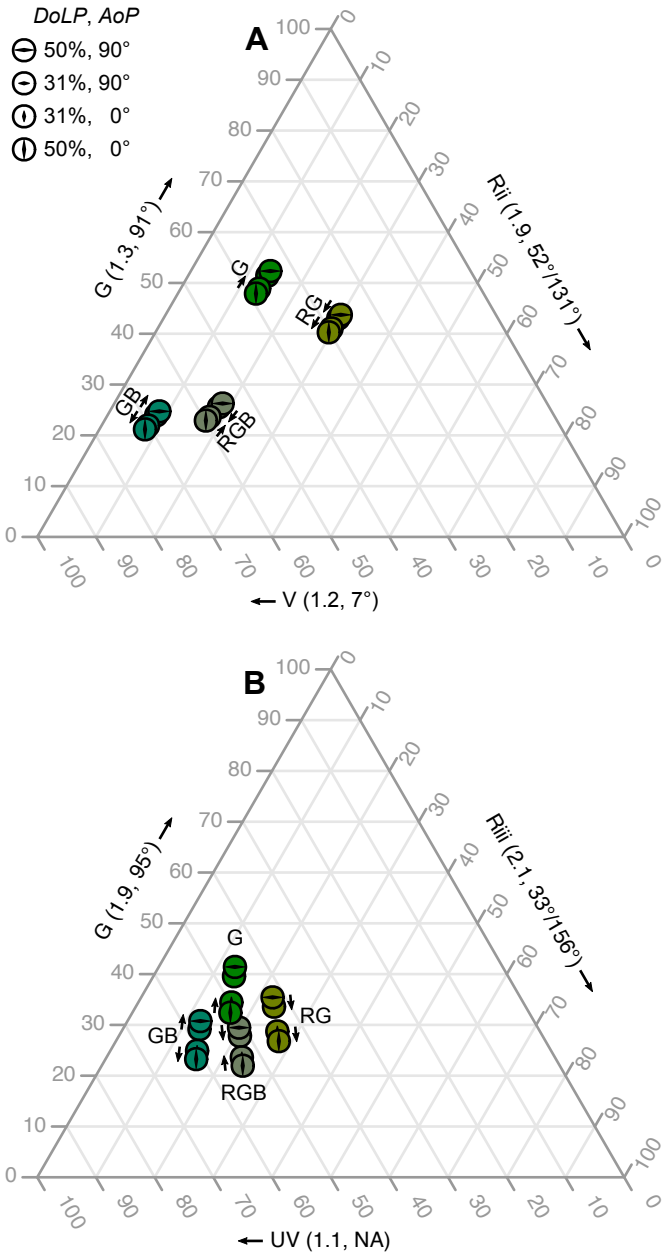

Fig. S2 Color triangles representing the modeled color space of *Pieris rapae* females. Triangles show a model of relative ultraviolet (UV), violet (V), green (G) and red photoreceptors' (Rii or Riii) quantum catch in type II (A) and III (B) ommatidia. The numbers in parentheses show the polarization sensitivity (PS) and axis of maximal polarization sensitivity (ϕ_{max}) of each receptor. The colored circles show the stimuli tested in the color-removal experiment. Arrows indicate the stimuli preferred by female *P. rapae*. DoLP, degree of linear polarization. AoP, axis of polarization.

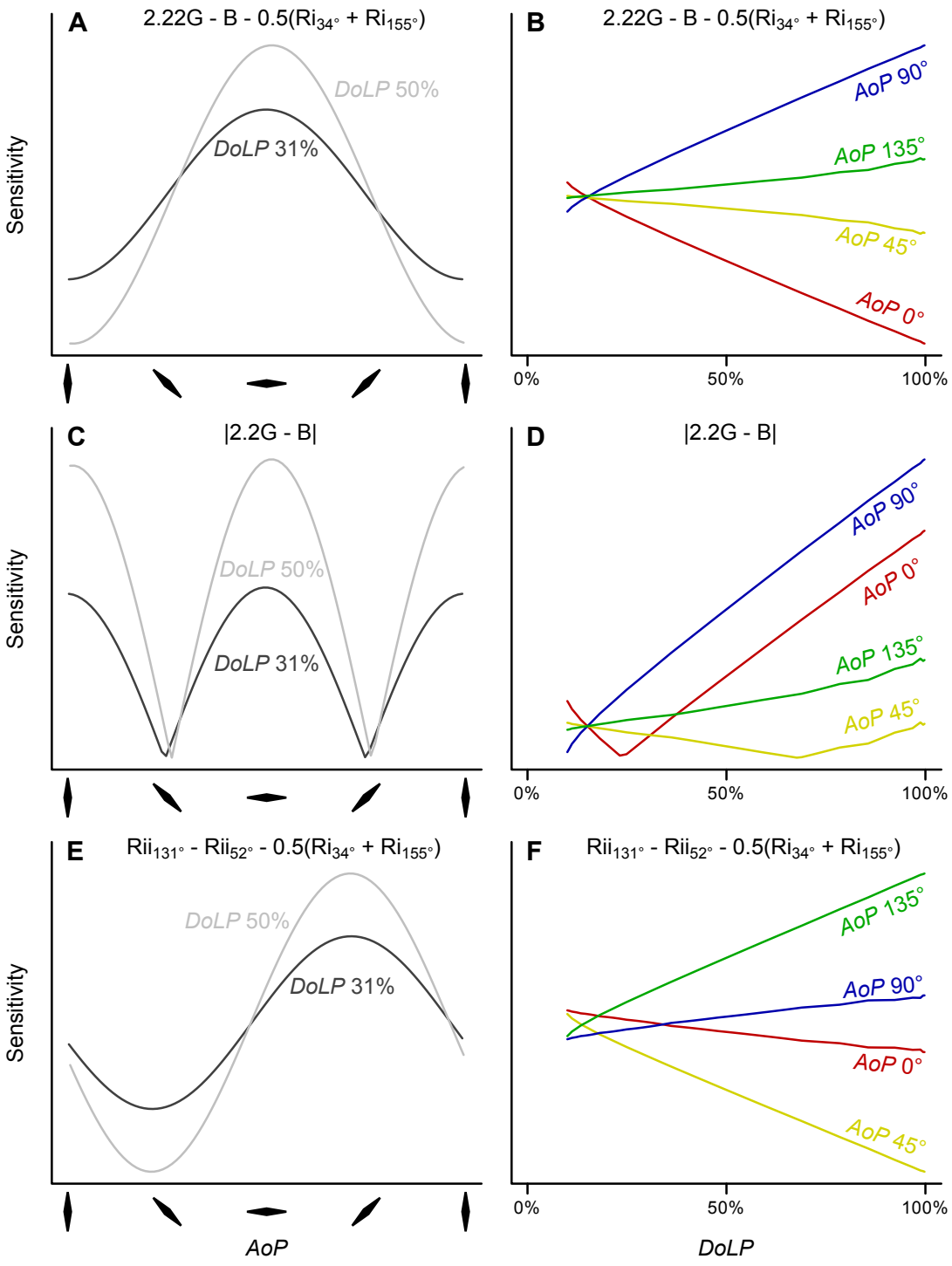

Fig. S3 Effect of AoP and DoLP image manipulations on models combining photoreceptor catch from *Pieris rapae* females. (A,C,E), Effect of AoP on models at DoLPs of 31% and 50%. (B,D,F), Effect of DoLP on models at AoPs of 0°, 45°, 90°, and 135°. (A,B), Color model involving red, green and blue photoreceptors from type I ommatidia (Fig. 5) which would also be representative of any comparisons between polarization-sensitive photoreceptors, or between one polarization-sensitive and one polarization-insensitive photoreceptor. (C,D), Model calculating the absolute difference between two photoreceptors. (E,F), Model comparing more than two photoreceptors. AoP = Axis of Polarization; DoLP = Degree of Linear Polarization