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Ecology

Color vision in *Lycaena* butterflies: Spectral tuning of receptor arrays in relation to behavioral ecology

(antihybridization/eyeshine/oviposition/territoriality/visual pigments)

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ABSTRACT **Males of two closely related, co-occurring species of *Lycaena* butterflies have dorsally blue (*Lycaena heteronea*) or red-orange plus ultraviolet (*Lycaena rubidus*) wings. Males are selectively territorial against conspecific males. Virgin females accept only conspecific males, probably chosen by wing color. Females are nonterritorial and spend most of their adult activity ovipositing on the correct larval food plants. Eyes of both species contain four spectral types of visual pigments (P360, P437, P500, and P568) but the distribution of these pigments within the receptor mosaic is quite different between both species and sexes. The ventral eye region of *L. heteronea* is tetrachromatic but that of *L. rubidus* is trichromatic, lacking the blue-sensitive visual pigment P437. The dorsal eye region of males of both species is dichromatic (P360 and P437). Visual-pigment spectra and wing-reflectance spectra are well matched for effective discrimination of wings of conspecific males from those of other species. The dorsal region of female eyes is trichromatic, containing P360, P437, and P568. The third visual pigment, P568, is important for long-range detection by ovipositing females of red coloration on *Eriogonum* and *Rumex* food plants. P568 has the same absorbance spectrum as the human red-cone and is considerably red-shifted compared to the P530 possessed by most insects. That the sexes and closely related species can have such major differences in distribution of visual pigments indicates that the visual system is as readily altered as wing coloration in the course of adaptive evolution.**

The Lepidoptera are one of the four large orders of insects, and their long evolutionary history has produced a wide diversity of families. These exhibit activity patterns that allow them to exploit many habitats, within the constraints of the phytophagous requirement for the larvae of most genera. Especially interesting is the development of diurnal adult activity in a few major lineages and the use of color that this biology makes possible. There is reason to suppose that the rapid evolution of diurnal insectivorous birds in the Cretaceous introduced powerful selection for nocturnicity among all Lepidoptera. Accompanied by substantial unpalatability to those new vertebrate predators, diurnicity has evolved at least 12 times among the extant Lepidoptera. Diurnicity permits the use of color signals on the wings or body in the behavioral ecology of any animal, and in butterflies both colors and the sensitivity to colors are richly developed.

Photochemical and physiological studies are revealing great interspecific diversity in the spectral properties of butterfly visual pigments and photoreceptors (1–3). Very little is known, as yet, about how chromatic information is processed by their visual systems (4). How do the sensory capabilities and limitations relate to the requirements of visually mediated behaviors?

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The "true butterflies" (Papilionoidea) exhibit some species clusters in which several close relatives are basically sympatric and seasonally synchronous. This must have imposed on them two evolutionary stresses at the beginning of sympatry, the tendency for gametic wastage during mate-choice errors and for niche competition for larval food plants (5).

We chose for the present studies on the evolution of color vision a complex of close relatives that we knew do not now hybridize, which utilize different larval foodplants as oviposition substrates and which have great differences in wing color between sexes and among species. These are the butterflies of the broad genus *Lycaena*, of which several species occur in easy reach of the Rocky Mountain Biological Laboratory in Gunnison County, Colorado, where we conducted the present research. Two particularly close species are *Lycaena heteronea*, which differs from its many congeners in having bright blue males, and *Lycaena rubidus*, which has vivid red-orange males like many other *Lycaena*. The females of both are grayish-tan dorsally and overlap extensively in color and pattern.

We characterized the spectral properties of their visual pigments and examined correlations to colors involved in territoriality, mate-finding, and food plant choice, in the zone of ancient sympatry.

The results of our extensive studies of male territorial behavior, courtship and copulation, and female oviposition of *L. heteronea* are being published elsewhere, with notes on four congeners. The field findings that are important for the present discussion of color vision are summarized as follows: Newly hatched females attract males by wind-carried pheromones, not visual signals. Flying males arrive, display their wing colors, and attempt to copulate, but the female accepts only a male with the conspecific coloration. After copulation the female spends the rest of her 2 weeks of adult life searching for the correct food plants (*Eriogonum subalpinum* or *Eriogonum umbellatum*) on which she glues eggs singly, several dozen per day. Males spend their adult life competing for suitable territory, presumably likely to yield newly hatching females. The alpha male on territory drives off conspecific males that approach within 1–3 m but ignores males of other *Lycaena* species and also the Blue *Plebejus icarioides*.

For both sexes, visual recognition of conspecific males is critical. On the other hand, recognition of larval food plants is critical for females but not males.

METHODS

Eyeshine created by tapetal reflectors makes the intact butterfly eye an excellent preparation for spectroscopic study of visual pigments. Consider the optics: The visual pigments are contained within a long, thin rhabdom waveguide that is terminated optically by a tapetal mirror. This spectroscopic sample of photopigments is illuminated efficiently by a pair of lenses and is wrapped in shielding pigment, protecting it from

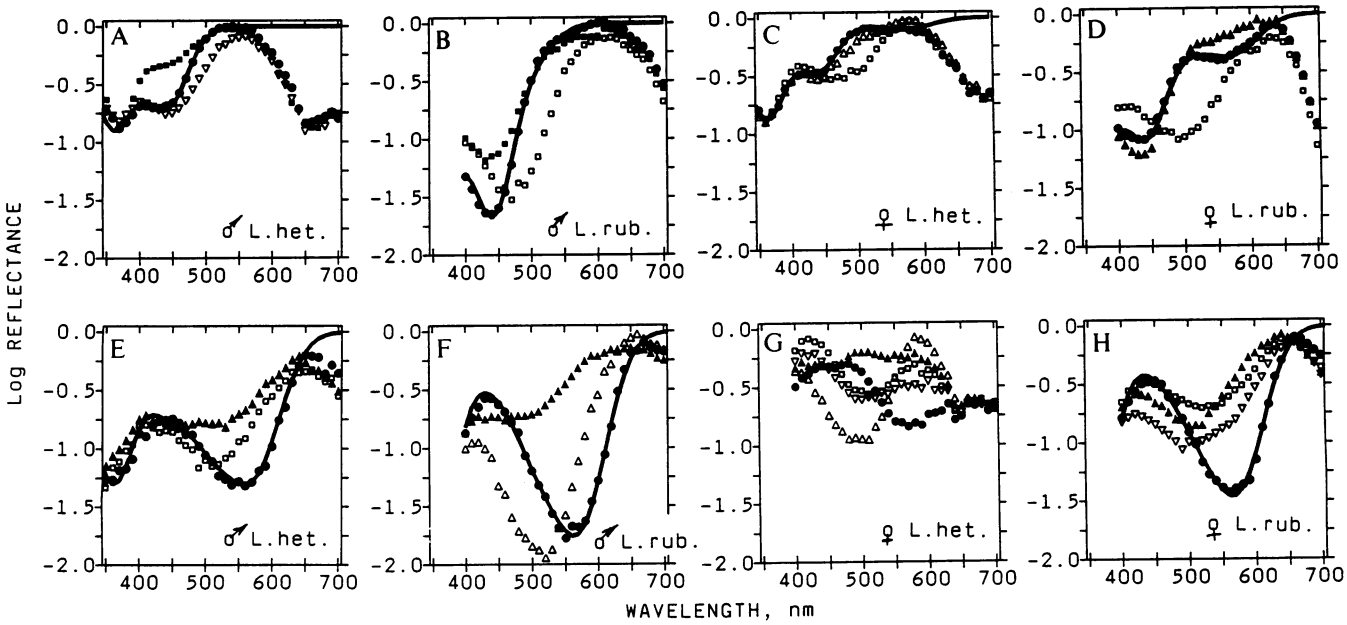

FIG. 1. Eyeshine reflectance spectra from dorsal (*A–D*) and ventral (*E–H*) eye regions. ●, Dark-adapted reference; after exposure to actinic red (△, ▲), then blue (□, ■), and then UV (▽). Open symbols for spectra were taken shortly after exposures; filled symbols for spectra were taken after photoproduct decay. Solid curves are least-squares fits of template absorbance spectra for the mixtures specified in Table 1.

stray light. The tapetal mirror is highly reflective over most of the visible spectrum. When an incident-light microspectrophotometer is focused for optimal collection of eyeshine, the photometer head measures light that has been tapetally reflected and survived a double pass through the rhabdom. Thus, the integrated, longitudinal absorbance of the rhabdom is proportional to one-half the common logarithm of the ratio: [measured reflectance/tapetal reflectance].

Photoisomerization of visual pigment creates a photoproduct ("metarhodopsin") that has a different absorption spectrum than the native pigment (6). Unlike many insects, in butterflies, metarhodopsin decays rapidly from the rhabdom in the dark. Thus, it is possible to measure *in vivo* the absorbance spectrum of a visual pigment by first recording the reflectance spectrum of the dark-adapted eye (the *reference spectrum*), then delivering actinic flashes, waiting in the dark for metarhodopsin to decay, and then recording the reflectance spectrum of the partially bleached rhabdoms (2).

Retinal Densitometry. The reference spectrum (Fig. 1, filled circles) of eyeshine reflectance was measured from a field of 10–20 ommatidia after 1 hr of dark-adaptation. Then the eye was exposed to periodic flashes of the *actinic red* light [45-W lamp filtered by Schott (Mainz, F.R.G.) RG630] to photoisomerize only the red-absorbing visual pigment. Reflectance spectra were measured before (Fig. 1, open triangles) and after (Fig. 1, filled triangles) waiting for metarhodopsin to decay from the rhabdom. Similarly, *actinic blue* [Ditric Optics (Hudson, MA) 415 nm] light was used to convert blue-absorbing pigment (Figs. 1 and 2, squares). In some experiments, *actinic UV* [Hoya (Fremont, CA) U360, inverted triangles] light was used to convert UV-absorbing pigment. See refs. 2 and 7 for additional details.

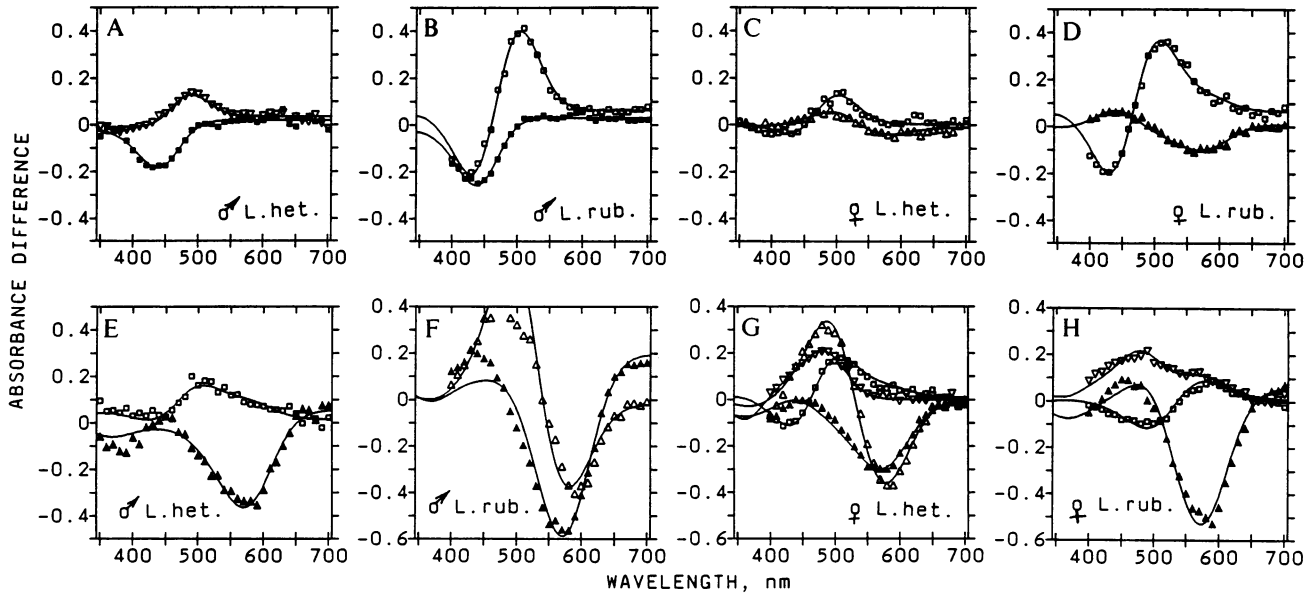

FIG. 2. Difference spectra from dorsal (*A–D*) and ventral (*E–H*) eye regions.

Table 1. Visual-pigment densities present in dark-adapted eyes, calculated from theoretical fits to reference spectra (●) of Fig. 1

Fig.	Species	Sex	Eye region	P360	P437	P500	P568	M495
1A	*L. heteronea*	♂	D	0.36	0.36	—	—	—
1B	*L. rubidus*	♂	D	0.50	0.77	—	—	0.15
1C	*L. heteronea*	♀	D	0.37	0.22	—	0.10	—
1D	*L. rubidus*	♀	D	0.51	0.48	—	0.18	0.08
1E	*L. heteronea*	♂	V	0.40	0.15	0.25	0.60	—
1F	*L. rubidus*	♂	V	0.50	—	0.25	0.83	—
1G	*L. heteronea*	♀	V	0.5	0.4	0.3	0.5	—
1H	*L. rubidus*	♀	V	0.60	—	0.17	0.70	—

D, dorsal: elevation = +40°. V, ventral: elevation = −30°.

Analysis of Eyeshine Spectra. Least-squares fits of template absorbance spectra (8, 9) to photochemical difference spectra (Fig. 2) were used to determine the wavelength for peak absorbance (*lambda-max*) of both visual pigment and metarhodopsin and the longitudinal one-way density of bleached visual pigment. Then each reference spectrum was analyzed to determine the average densities of visual pigments contained in the dark-adapted eye (Table 1), assuming that the tapetal reflectance spectrum is spectrally flat.

RESULTS

Retinal Densitometry and Photochemistry. Analysis of difference spectra (Table 2) revealed (*i*) an unusually red-sensitive visual pigment (P568), with lambda-max of 568 nm; (*ii*) a blue-sensitive P437; (*iii*) metarhodopsins (M495) of both P568 and P437, with lambda-max of 495 nm; and (*iv*) a UV-sensitive P360 with metarhodopsin M480 (see Fig. 3).

Destructive interference within the multilayered tapetal reflectors causes eyeshine reflectance to drop at long wavelengths; tapetal reflectance is constant at shorter wavelengths. However, shielding of the whitish tapetum by visual-pigment absorption causes the dips in reflectance spectra (Fig. 1) at approximately 360 nm, 437 nm, and 568 nm, the size of which depends on the pigment densities. The heavy lines of Fig. 1 *A–H* are analytical fits of experimental reference spectra by theoretical pigment mixtures, the densities of which are given in Table 1.

Analysis of ventral spectra (Fig. 1 *E–H*) revealed the presence of a fourth class of visual pigment, P500, not present dorsally (Fig. 3). P500 exhibits no spectral shift upon photoconversion. Precedence for this type of pigment was established in another lycaenid butterfly, *Apodemia mormo* (3).

Wing Reflectance. Reflectance spectra from dorsal wing surfaces of *L. heteronea* and *L. rubidus* (Fig. 4*A*) bear an interesting relationship in which the maximum of one is positioned at a minimum of the other. The spectrum from a sympatric Blue *P. icarioides* is shifted to considerably shorter wavelengths compared to the blue-green peak of *L. heteronea*. Spectra for the grayish ventral surfaces (Fig. 4*C*) are all similar.

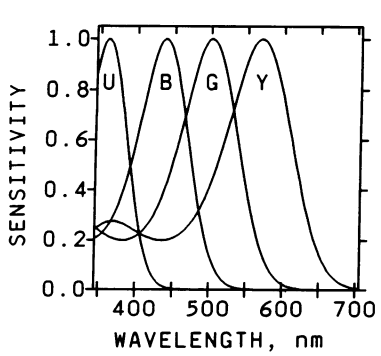

FIG. 3. Normalized spectral sensitivity functions of the four spectral types of receptor (U, containing P360; B, containing P437; G, containing P500; and Y, containing P568).

Plant Reflectance. The *Eriogonum* host plants of *L. heteronea* have red coloration on stems and umbels as well as many red leaves mixed among the green. Reflectance of this red pigmentation is much higher at long wavelengths than that of a green leaf (Fig. 5*A*).

DISCUSSION

Eyes of these two species of *Lycaena* contain the same four visual pigments (Fig. 3), but there are great differences among both species and sexes in how those pigments are distributed within the receptor mosaic (Table 1). In the dorsal eye region, both males have only P360 and P437, whereas females have P360, P437, and P568. In the ventral region for both sexes, *L. heteronea* has all four pigments, whereas *L. rubidus* lacks P437.

How well suited are these systems of photoreceptors for the job of discriminating among the various wing colors? We approach this question by calculating the optical stimulation (quantum catch, *Q*) of each receptor type viewing a daylight-illuminated wing (10, 11):

$$Q = \int Q_0(\lambda)R(\lambda)S(\lambda)d\lambda,$$

Table 2. Densities of pigment loss and photoproduct gain caused by actinic exposures, based on difference spectra of Fig. 2

Fig.	Actinic exposure	Time in dark, min	P360	P437	P568	M495	M480
2A, ■	Blue*	17	—	0.20	—	—	—
2A, ▽	UV	1	0.04	−0.10	—	—	0.13
2B, □	Blue	4	—	0.43	—	0.40	—
2B, ■		60	—	0.29	—	—	—
2C, △	Red	1	—	—	0.04	0.08	—
2C, □	Blue	1	—	0.09	—	0.13	—
2D, ▲	Red	12	—	−0.08	0.11	—	—
2D, □	Blue	4	—	0.40	−0.07	0.33	—
2E, ▲	Red	30	—	—	0.42	—	—
2E, □	Blue	1	—	0.06	−0.08	0.12	—
2F, △	Red	13	—	—	0.41	0.80	—
2F, ▲	Red*	15	—	—	0.80	0.09	—
2G, △	Red	2	—	—	0.40	0.48	—
2G, ▲		50	—	−0.06	0.29	—	—
2G, □	Blue	2	—	0.20	−0.05	0.18	—
2G, ▽	UV	2	0.12	—	—	—	0.21
2H, ▲	Red*	29	—	—	0.62	0.20	—
2H, □	Blue	1	—	—	−0.10	−0.15	—
2H, ▽	UV	2	0.1	—	−0.10	—	0.17

*Overnight exposures.

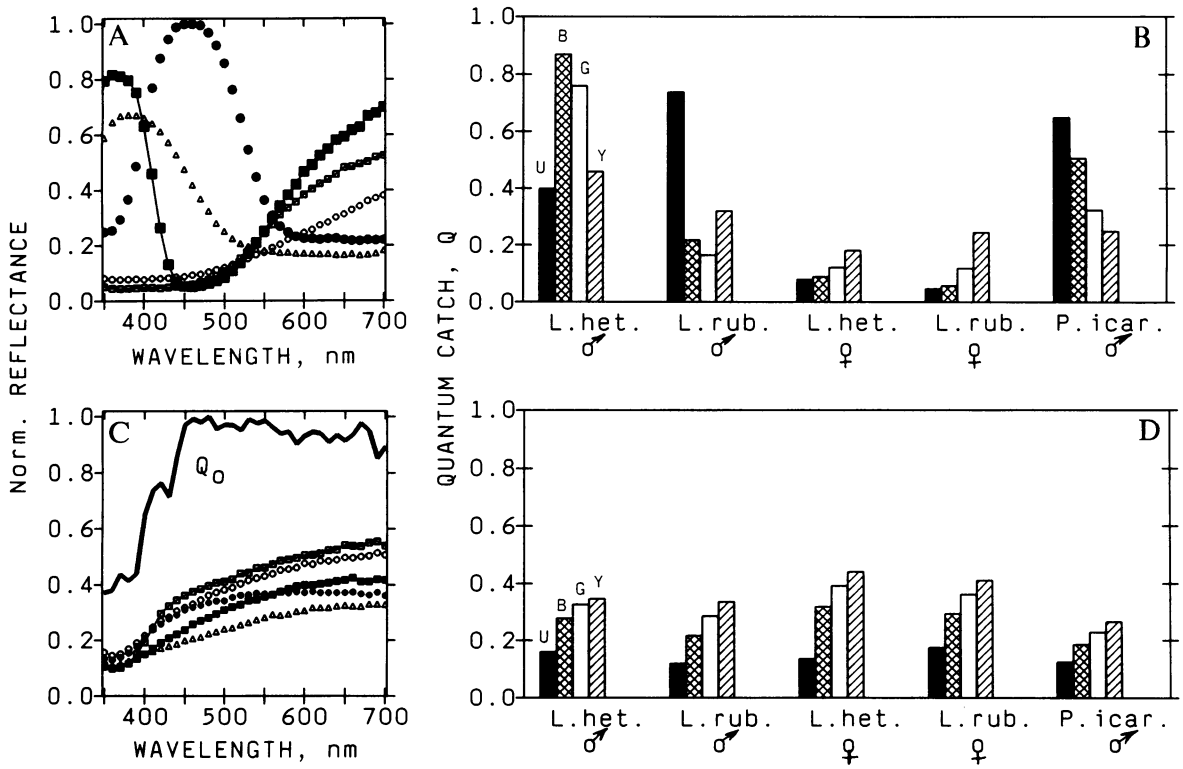

FIG. 4. (*A*) Reflectance spectra for dorsal wing surfaces of *L. heteronea* male (●) and female (○); *L. rubidus* male (■) and female (□); and *P. icarioides* male (△). (*B*) Optical stimulation of each receptor type viewing the dorsal wing surfaces specified on the abscissa. (*C*) Reflectance spectra for ventral wing surfaces; Q_0, normalized quantum flux of daylight. (*D*) Optical stimulation of each receptor type viewing the ventral wing surfaces specified on the abscissa.

where $Q_0(\lambda)$ is the quantum flux of daylight illumination (10) (see Fig. 4*C*), $R(\lambda)$ is the wing's reflectance spectrum, and $S(\lambda)$ is the spectral sensitivity function for a single photoreceptor cell (approximated by the absorptance spectrum of the cell's visual pigment, Fig. 3). Bar graphs of Q, normalized to unity for an ideal flat-white object, are shown in Fig. 4 *B* and *D* for each wing.

In male eyes, the dorsal region is blind in the red/orange/yellow but has the capability for dichromatic color vision between 300 nm and 500 nm using receptors ("type-U") that contain P360, and receptors ("type-B") that contain P437. We observed territorial flights by male *L. heteronea* initiated while perched in a head-down posture. This indicates that the dorsal eye region of males is sufficient to mediate territorial behavior. Thus, we hypothesize that this behavior is driven by a dichromatic color vision system.

Color differences judged by a dichromatic system depend on the ratio of Q values for the two receptor types (11). Q_B/Q_U for male wings is largest for *L. heteronea*, smallest for *L. rubidus*, and close to unity for *P. icarioides* (Fig. 4*B*).

How well matched are the spectral properties of eyes and wings, for best discrimination among sympatric males? The UV peak of *L. rubidus* wings is very well matched to the type-U sensitivity function. The blue-green peak of *L. heteronea* and null of *L. rubidus* wings are so broad that lambda-max of the type-B receptor could be anywhere between 437 nm and 520 nm and still have good separation of stimulation ratios. Thus, a dichromatic system involving P360 and P500 would also perform well.

Visual pigment P568 has the same absorbance spectrum as the human red-cone and is considerably red-shifted compared to the P530 possessed by most insects. It may be useful to ovipositing females for long-range detection of host plants. As shown in Fig. 5*B*, shifting from visual pigment P530 to P568 increases by 57% the stimulation of receptors viewing red leaves.

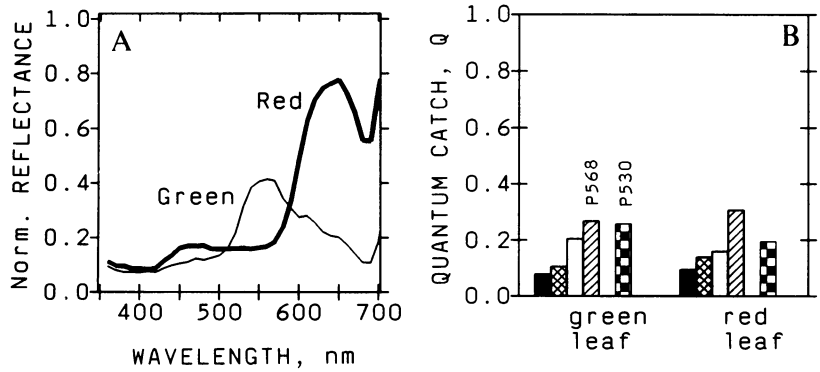

FIG. 5. (*A*) Reflectance spectra for red and green leaves of *E. subalpinum*. (*B*) Comparison of optical stimulation of each receptor type to one containing P530.

The presence of visual pigment P500 is important for color vision, because it enables excellent hue discrimination over the range 440–600 nm through opponent processing (10, 11) B–G and G–Y. This does not necessarily imply that butterflies possess "true" color vision as demonstrated for humans and worker bees (12).

Our study indicates that (*i*) visual decisions in the color-oriented behavior of butterflies may be guided largely by particular combinations of receptor types within the species and sex-specific receptor mosaic; and (*ii*) differences in these mosaics, as well as the correlated behaviors, can change readily during the evolution of the antihybridization and anticompetition systems essential to stable sympatry of close relatives.

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