J Comp Physiol A (1988) 162:23–33

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Modification of spectral sensitivities by screening pigments in the compound eyes of twilight-active fireflies (Coleoptera: Lampyridae)*

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Accepted June 23, 1987

Summary. 1. ERG S(λ) were determined in dark-adapted intact preparations of 6 North American firefly species (*Photinus collustrans, marginellus, pyralis, macdermotti, scintillans* and *Bicellonycha wickershamorum*) which restrict their flashing activity to twilight hours. The curves possess narrow (1/2 bandwidth = 50–60 nm) peaks in the yellow (560–580 nm) and a shoulder in the violet (370–420 nm), with a *marked* attenuation (1.4–2.2 log units) of sensitivity in the green (480–530 nm) region of the spectrum (Fig. 1). Two additional species (*Photuris potomaca* and *frontalis*) which initiate flashing at twilight and continue on late into the night (twi-night) possess broad sensitivity maxima around 560 nm (Fig. 3).

2. Selective adaptation experiments isolated near-UV and yellow in *P. scintillans* (Fig. 2). In the dorsal frontal region of the compound eyes in *P. frontalis*, high sensitivity existed only in the short wavelength region (near-UV and blue) with a maximum in the blue (λ_{max} 435 nm) (Fig. 4).

3. The in situ MSP absorption spectrum of the screening pigments was determined in preparations of firefly retina. a) Two kinds of dark brown granules were found in the clear zone region. These granules absorb all across the spectrum with a gradual increase in optical density in the shorter wavelength region in *P. pyralis* (Fig. 5). b) Besides dark granules, pink-to-red colored screening pigments were present in the vicinity of the rhabdoms. The absorption spectra of these pigments determined in five species were narrow (1/2 bandwidth = 50–80 nm) with species-specific differences in their peak absorption in the green at 525 nm, 510 nm, 512 nm and 517 nm in *P. scintillans, macdermotti, collustrans* and *pyralis*, respectively (Fig. 6). A similar pigment was found in *P. marginellus* with a λ_{max} at 512 nm (Fig. 7). In all cases, transmission increased both at long and short wavelengths, but more sharply in the long wavelength region (Figs. 6 and 7). Hence each twilight-restricted species has its own unique colored screening pigment. A yellow pigment whose absorption spectrum differed from those found in genus *Photinus* was found in twi-night active *Photuris potomaca* (λ_{max} 461 nm) and night-active *P. versicolor* (λ_{max} 456 nm). The transmission of the *Photuris* pigment increased sharply only in the long wavelength region (Fig. 8).

4. In the twilight-restricted species, pink-to-red screening pigments modify dramatically the long green wavelength part of S(λ) functions. The calculated effect of the absorption of these screening pigments (O.D. = 1.6 to 2.2; $\bar{X} = 1.8$, $n = 4$) on a theoretical S(λ) curve represented by a green (P550) rhodopsin, match the shape of the experimentally obtained dark-adapted ERG S(λ) in all cases (Figs. 9, 10). These screening pigments (Figs. 6, 7, 8) then would cause attenuation of sensitivity selectively in the green in twilight-restricted fireflies (Fig. 1) with a concomitant shifting of the peak of the sensitivity in the yellow as well as a narrowing of the visual spectral sensitivity. The pink-to-red colored screening pigments presumably would enhance color and/or brightness contrast in the mesopic range of ambient illumination.

5. The presence of the colored screens attenuates absolute sensitivity from 5–25% among differ-

Abbreviation: MSP microphotospectrometer

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ent twilight-active species as compared to a night-active *Photuris lucicrescens* (Fig. 11).

Introduction

The electroretinographic (ERG) spectral sensitivities S(λ) possess narrow peaks in the yellow with a marked (1.0–2.0 log units) reduction in sensitivity in the green in twilight-active fireflies: *Photinus pyralis* (λ_{max} 565 nm) and *P. scintillans* (λ_{max} 565 nm), whereas broad peaks in the green are found in the dark-active species, *Photuris versicolor* and *P. lucicrescens* (λ_{max} 565 nm) (Lall 1981a, b; Lall et al. 1980a, b, 1982; Seliger et al. 1982b). It is of interest to know whether the shape of visual spectral sensitivity S(λ) observed in *P. pyralis* and *scintillans* occurs generally among twilight-active fireflies. Therefore S(λ) was determined in six more species which initiate their luminescence flashing activity at twilight. We also were interested in the physiological mechanisms which cause attenuation of visual sensitivity in the green region of the spectrum, resulting in narrow peaks in the yellow. Since in *P. pyralis* the spectral sensitivity of the lamina potential in preparations devoid of the distal screening pigment granules was similar to the corneal ERG S(λ) in intact preparations, the distal screening pigment granules could not be implicated in causing attenuation (about 1.5 log units) of sensitivity selectively in the green. Hence the presence of colored screening pigment near the rhabdomeric region was proposed for attenuating visual sensitivity in the green (Lall 1981b). Recently, in our quantitative optimization model of vision for the detection of bioluminescence (Seliger et al. 1982a), we hypothesized that all fireflies possess a green rhodopsin (P550) system which can be approximated by an Ebrey and Honig (1977) nomogram and that, in addition, the twilight-active firefly species also contain pink to red photostable screening pigment(s) absorbing in the green. The overlying of such a photostable screening pigment over P550 rhodopsin would effectively attenuate visual sensitivity in the green and would cause a shift in the peak of the S(λ) toward longer wavelengths, narrowing the S(λ) curves.

The purpose of this paper is to present S(λ) in six additional species (besides *P. pyralis* and *scintillans*) which initiate their flashing activity at twilight. In addition, we present in situ MSP absorption spectra of screening pigments from the accessory pigments sleeves in *P. pyralis* and the photostable yellow-to-red colored screening pigments for 7 species. The latter confirm our earlier hypothesis (Seliger et al. 1982a) that indeed screening pigment(s) attenuate visual sensitivity in the green. Preliminary reports of our findings have been presented (Lall et al. 1984, 1985).

Materials and methods

Animals. Fireflies (males) of the species *Photinus pyralis, marginellus, macdermotti, scintillans* and *Photuris frontalis* were collected at twilight in the Baltimore area, *P. collustrans* in Gainesville, Florida; *Bicellonycha wickershamorum* in Arizona, and *Photuris potomaca* at Point of Rocks, W. Virginia during the summer months (June to August) from 1979 to 1984. The collected insects could be maintained at room temperature for several days. In the field, the males were identified by their species-specific temporal patterning of the flash signals.

Optical system. The optical system utilized in these experiments has been described in detail earlier (Lall et al. 1980a) and is only summarized here. Our system contains one test and two adaptation light beams, all with quartz optics, independently variable in wavelength and intensity. The beams were finally combined by beam splitters into one single beam which was focused on the entrance of a quartz light pipe (3 × 36 nm) by a collecting lens. The corneal surface of the eye was completely illuminated by the light coming out of the end of the light pipe. A series of neutral density filters attenuated the intensity of the light in the test beam over 6 log units. A Uniblitz shutter regulated the stimulus duration.

Light from a 150 W xenon arc lamp operated at 7.5 A using a regulated power supply passes through a Bausch and Lomb high intensity monochromator (bandwidth 9.9 nm) to provide the test beam. The stray light was eliminated by placing a series of Corning glass colored filters in the light beam. The two adaptation beams were provided by another 150 W xenon arc lamp and a 500 W tungsten light source. Interference and Corning glass filters were used for chromatic adaptation experiments. The quantum flux of the test beam was determined by using a calibrated PIN 10/UV photodiode (United Detector Technology, Inc., Santa Monica, CA), which was periodically calibrated against an Eppley linear junction thermopile whose output was read on a Keithley microammeter.

Electrical recordings and experimental procedures. Details of the animal preparation and experimental procedures have been described earlier (Lall et al. 1980a). ERGs were recorded by a glass pipette (resistance <1 MΩ, 2–5 μm in tip diameter) filled with insect Ringer's solution and positioned subcorneally. For reference, another glass electrode was placed in the antennae. Ag-AgCl wires connected the electrodes to the input of a high impedance preamplifier (Grass P16) whose output in turn was fed to a CRO and an ink writer. The face of the CRO could be photographed.

The eye was dark-adapted for 40 min to an hour prior to experimentation. Light flashes of varying durations (50–400 ms) at test wavelength were administered over 6 log unit change in stimulus intensity. The intensity of light needed to elicit a criterion amplitude (2.5 mV) was determined at various test wavelengths across the spectrum (340–700 nm). A plot of 1/log Q as a function of wavelength gave the spectral sensitivity curves. The experiments were performed during late afternoon and evening at room temperature (21–23 °C).

Microspectrophotometric measurements. Two different instruments in two different laboratories were used in these experi-

ments. A simplified recording microspectrophotometer previously described (Casella et al. 1975) was used in the first set of experiments. A continuous chart record of the extinction over the wavelength range 400–600 nm was obtained in about 20 s. The numerical aperture was 0.3, the measured area being 3.12 μm in diameter and the entering half-bandwidth was 6.6 nm. An external reference beam is used in this instrument; the extinction values as a function of wavelength refer to a clear area of the preparation and are plotted manually from the recorder charts, taken every 10 to 20 nm over the spectrum.

The evaluation of the instrumental losses due to glare or reflection was made by measuring the density of a small opaque object and spots of black spray paint with a diameter about 8 μm. The optical density values obtained ranged from 1.9 to 2.1 for the paint spots; while those for a graphite granule of diameter 1–2 μm, the optical density value was 1.6 units.

The details of the second MSP instrument are given in a recent paper (Cronin 1985). It consisted of a single-beam design. Light from a 100 W tungsten-halogen source was passed through a grating monochromator equipped with a stray light filter and operated with entrance and exit slit set at 0.4 mm (half-band width ≅ 4 nm). The beam continued through a small field diaphragm, a linear polarizer and an electromagnetic shutter. The beam was then deflected into the microscope by a beamsplitter, and the image of the monochromater slit limited in length by the field diaphragm was demagnified and focussed onto the specimen by a Nikon 100× UV-F glycerin-immersion objective (numerical aperture = 0.8). Beam dimensions in the specimen plane were 0.5 × 2 μm, with the plane of polarization parallel to the long axis. Transmitted light was collected by an identical 100× objective, and measurements were made using a PARC 1140 photometer coupled to an IBM PC.

Preparation. The absorption spectrum of the pigments was obtained in fresh (unfixed) firefly eyes either by cutting thin slices of the retina with a vibrating razor blade or by teasing apart the retina with fine needles. The samples were transferred to a microscope cover slip in insect Ringer's solution. A second cover slip sealed with stopcock grease completed the preparation for viewing on the stage of the microspectrophotometer (MSP).

Results

ERG S(λ) functions

The ERGs recorded in the six species described here were typical on-negative potentials and have been described in detail for *P. pyralis* earlier (Lall et al. 1980a). The slope of the V/logI curves for different stimulus wavelengths across the spectrum were similar among different species and the eye responded over 4 to 5 log units of change in the stimulus intensity, as has been described earlier for three species (Lall et al. 1980a; Lall 1981a; Lall et al. 1982).

The average S(λ) functions for a criterion response (2.5 mV) elicited by dim photic stimulus obtained from the dark-adapted compound eyes in the males of 6 species of fireflies which restrict their flashing activity to a short period at twilight (twilight-restrictive) are presented in Fig. 1. All curves possessed two peaks of maximal sensitivity, one in the yellow (λ_{max} range 560 to 580 nm) and a second in the short wavelengths (λ_{max} range 370 to 420 nm) with a marked attenuation of sensitivity in the green region of the spectrum. The sensitivity dropped precipitiously between 515 and 535 nm and then rose 1.5 to 2.2 log units in less than 30 nm in all cases, resulting in an unusually narrow peak

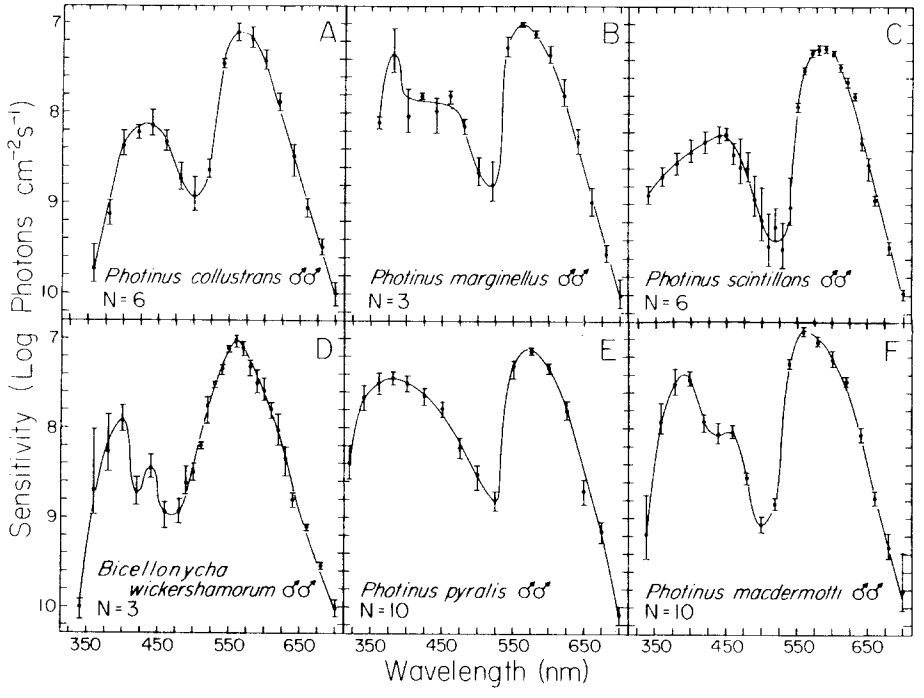

Fig. 1. ERG S(λ) from dark-adapted compound eyes in twilight-restricted species: *Photinus collustrans* ♂ (**A**), *P. marginellus* ♂ (**B**), *P. scintillans* ♂ (**C**), *Bicellonycha wickershamorum* ♂ (**D**), *P. pyralis* ♂ (**E**) and *P. macdermotti* ♂ (**F**). Log photons cm^{-2} s^{-1} required to produce 2.5 mV criterion amplitude of the on-negative ERG is plotted on the ordinate. Bars ±1 SE of the mean

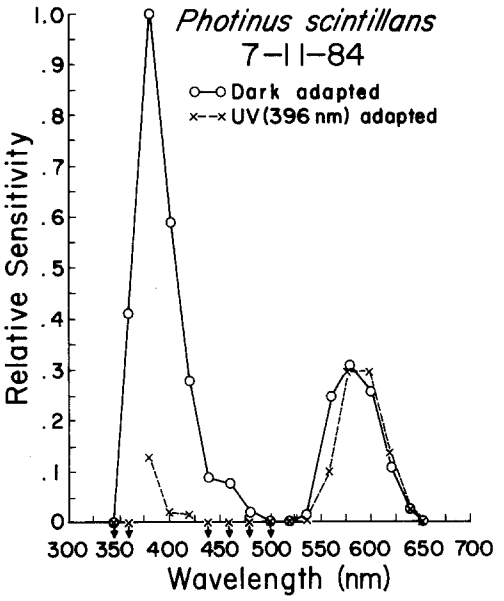

Fig. 2. Comparison of ERG S(λ) between a dark-adapted curve and under selective adaptation with 390 nm light. The adaptation effect was 2.9 log units. The curves are superimposed for clarity. Recordings from the dorsal frontal margin in *P. scintillans* ♂ compound eyes. Downward arrows indicate wavelengths at which the criterion value (0.5 mV) could not be obtained. Data from one animal

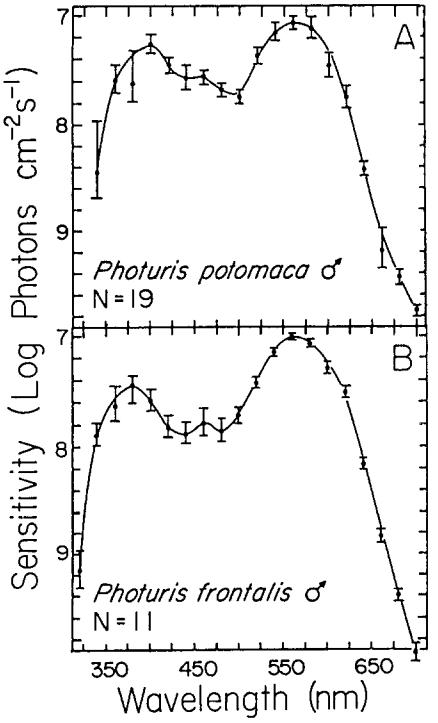

Fig. 3. ERG S(λ) curves (for 2.5 mV criterion amplitude) from dark-adapted compound eyes in the males of twi-night active species: *Photuris potomaca* ♂ **(A)** and *Photuris frontalis* ♂ **(B)**. Bars ± 1 SE of the mean

(1/2 bandwidth about 50 nm) in the yellow region of the spectrum. In the 6 species studied here, the maximum in the yellow is at 560 nm, 565 nm, 580 nm, 560 nm, 570 nm and 575 nm in *Photinus collustrans, marginellus, scintillans, B. wickershamorum, P. pyralis* and *macdermotti*, respectively (Fig. 1A, B, C, D, E and F).

In *P. scintillans* the S(λ) curve from the dorsal-frontal region of the compound eyes possessed an unusually high sensitivity in the short-wavelength region (Fig. 2). This short wavelength sensitivity was reduced to a residual level with high intensity 390 nm selective adaptation. Similar selective adaptation with intense violet and yellow lights in *P. pyralis* retina (Lall et al. 1980a) reduced sensitivity in the short (near-UV and violet) and in the long (yellow) wavelength region, respectively. Together these two studies suggest the presence of at least two receptor types in the compound eyes of *P. pyralis* and *scintillans*. Similarly the 4 other firefly species could also possess at least two receptor types. The height of the short wavelength sensitivity varied as a function of the positioning of the corneal electrode. In the experiments reported in Figs. 1 and 3, the recording electrode was placed in the medial equitorial region of the eye to reduce variability among data sets.

Besides the twilight species, there exist a number of species which initiate flashing at twilight and continue their flashing activity late into the night (twi-night species). Two species, *Photuris potomaca* and *frontalis*, belonging to the latter group were investigated. The wavelength maxima of S(λ) functions exist at 560 nm, with only *some* attenuation of sensitivity in the green (Fig. 3).

In *P. frontalis* eyes, when the recording electrode was placed in the dorsal frontal margin, the S(λ) possessed a maximum in the short wavelengths, (Fig. 4) without any contribution in the long wavelengths. Ebrey and Honig's (1977) nomogram for P435 fits the blue section of the curve, and high sensitivity also exists in the near-UV. Earlier, in *P. lucicrescens*, a blue (λ_{max} 435 nm) and a near-UV mechanism were isolated by selective adaptation experiments (Lall et al. 1982). The presence of only blue and near-UV receptors is strongly suggested in the dorsal frontal region of the compound eyes (Fig. 4).

In situ MSP absorption spectra of screening pigments

Pigment granules of the distal sleeve. The absorption spectra of the granules located in the pigment

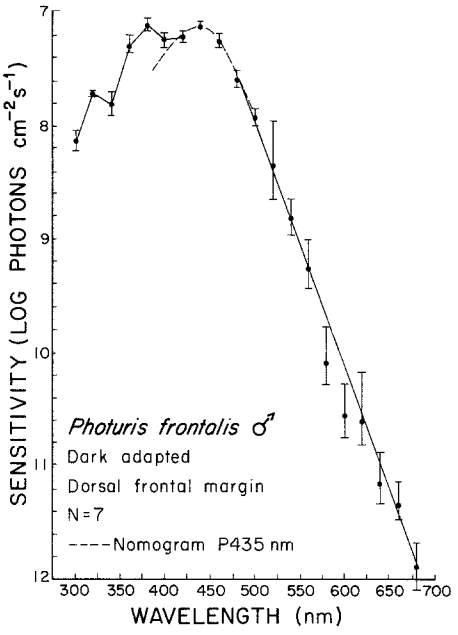

Fig. 4. $S(\lambda)$ functions (for 2.5 mV criterion amplitude) obtained from the dorsal frontal region of the compound eyes in dark-adapted *Photuris frontalis* ♂. Bars ±1 SE of the mean. Dashed line represents Ebrey and Honig (1977) nomogram curve for P435

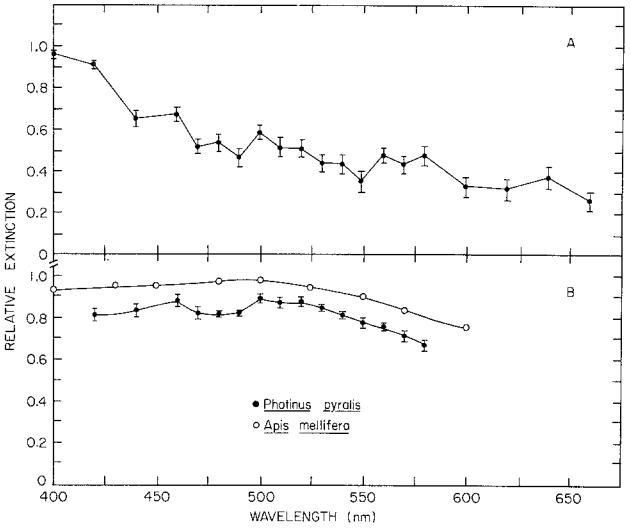

Fig. 5. Normalized absorption spectrum of the screening pigment located in the clear-zone region of the compound eye in *Photinus pyralis* ♂ Curve **A** ($N=7$) absorption spectrum of the dark brown pigment granules, curve **B** ($N=10$) of dark reddish grown granules (•). Bars ±1SE, or the mean. **B**, open circles dark red screening pigment absorption spectrum from the eye of the worker honeybee, *Apis mellifera* (Fig. 8 in Strother and Casella 1972). The spectra are displaced vertically to facilitate comparison

cells of the clear-zone region distal to the rhabdomeric segment are presented in Fig. 5. Under visual observation, the color of these pigment granules varied from dark brown to reddish dark brown in the fresh preparation. a) The extinction of the dark brown granules is very broad with a gradual rise in the short wavelength region below 450 nm (Fig. 5A). On the long wavelength side there is a gradual decline in absorption above 520 nm. b) The dark reddish brown granules possess high absorbance all across violet, blue and green yellow wavelength with a decrease in the red region (Fig. 5B). This pigment is nearly identical to the worker honeybee dark red screening pigment reported earlier (see Fig. 8 in Strother and Casella 1972). This leads one to believe that the red brown pigment may be of general occurrence among insect eyes. The flatness of the absorption spectra of the distal screening pigment granules (Fig. 5) confirm our earlier hypothesis based on electrophysiological data (Lall 1981b).

Colored pigments. a) A light magenta red or pink screening pigment of high viscosity was observed in the vicinity of the rhabdomeric region of the compound eyes in twilight-restrictive fireflies. The peaks of the absorption spectra of these colored pigment screens in 4 species of fireflies are at 525 nm, 510 nm, 512 nm, and 517 nm in *Photinus scintillans, macdermotti, collustrans,* and *pyralis* respectively (Fig. 6). In *P. marginellus* the pink pigment's λ_{max} is at 512 nm (Fig. 7). The absorption spectra are unusual since they are asymmetric and show a sharp peak in the green region. The drop-off in absorption is much more pronounced in the long wavelength than in the short wavelength region.

b) A yellow pigment was found in the clear-zone region in *Photuris potomaca* and *P. versicolor* compound eyes and its absorption spectrum is high in the blue with peaks at 461 nm and 465 nm, respectively, with a sharp fall in the long wavelengths (Fig. 8). The absorption of this yellow pigment (Fig. 8) differs markedly from the absorption spectra of the pink-to-red pigments presented in Figs. 6 and 7. This absorption spectrum of the *Photuris* pigment (Fig. 8) is very similar to one obtained in the oil droplets of turtle retina and may represent a zeaxanthin (3:3′-dihydroxy-β-carotene) pigment (Lipetz 1984).

Modification of visual spectral sensitivity by screening pigments – a comparison between the model and the observed data

One of the assumptions of our optimization model is that the green visual pigment (P550), whose absorption spectrum can be approximated by an Ebrey and Honig (1977) nomogram, mediates

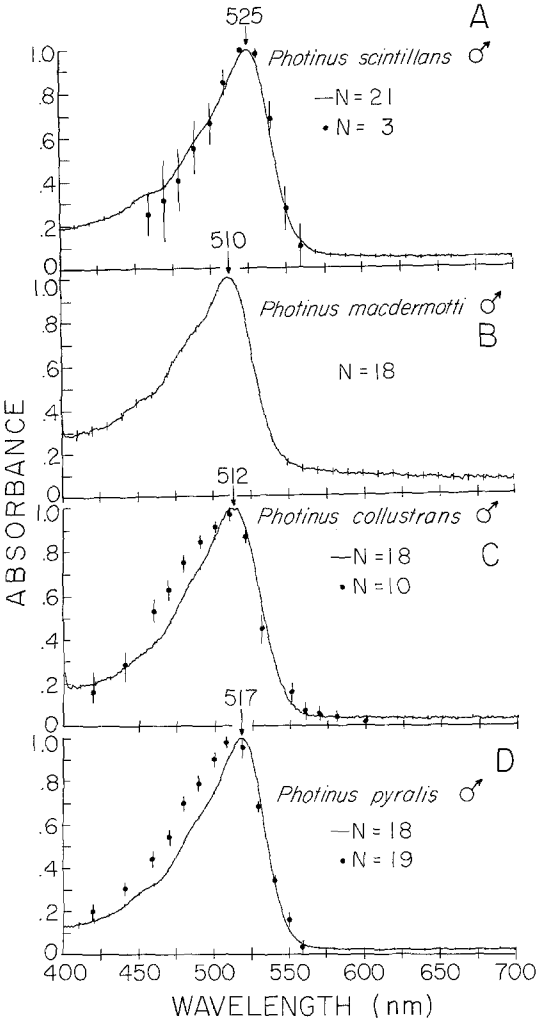

Fig. 6. Normalized absorption spectrum of the orange to red pigment located in the rhabdomeric slices of the compound eyes in the males of the firefly species: *Photinus scintillans* ♂ (**A**), *P. macdermotti* ♂ (**B**), *P. collustrans* ♂ (**C**), and *P. pyralis* ♂ (**D**). Data obtained in Cronin's lab (continuous line) are in good agreement with those obtained in Strother's lab (only points are given). Bars ±1 SE

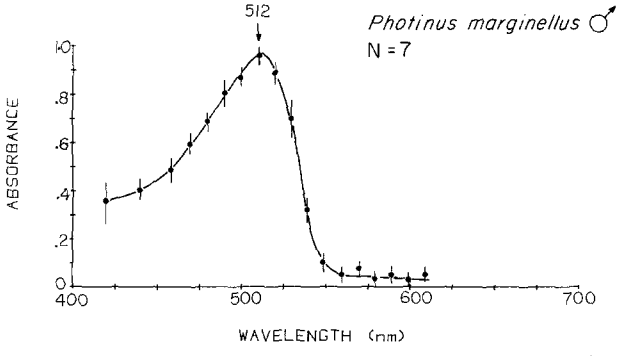

Fig. 7. Normalized absorption spectrum of orange screening pigment from the compound eye in *P. marginellus* ♂

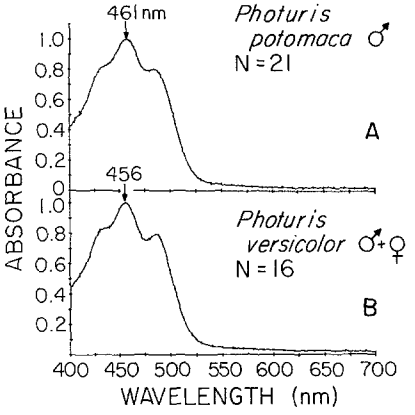

Fig. 8. Normalized absorption spectrum of the yellow screening pigment from the compound eyes in *P. potomaca* ♂ (**A**) and *P. versicolor* ♂+♀ (**B**)

vision in the long wavelength region in nocturnal fireflies (Lall 1981a; Seliger et al. 1982a). When a colored photostable pigment (different from distal screening pigment granules described in Fig. 5) is interposed in front of the visual pigment, the effective absorption is different than that of the visual pigment alone, such that absorption is markedly attenuated in the region where the absorption of the screening pigment is maximized. The modification of the absorption of P550, when it is overlaid by a species-specific photostable colored screening pigment, is calculated in Figs. 9A, B, C and D. Goldsmith (1978), Goldstein and William (1966) have considered the theoretical effect of such an inert screening pigment. When a receptor with rhodopsin absorbance $D(\lambda)$ is overlaid by a screening pigment with absorbance $D'(\lambda)$ then the absorptance (or fraction absorbed) of the rhodopsin $S'(\lambda)$ is given by

$$S'(\lambda)=10^{-D'(\lambda)}[1-10^{-D(\lambda)}]. \quad (1)$$

The function $10^{-D'(\lambda)}$ is the transmittance of the overlying pigment filter, and the function $[1-10^{-D(\lambda)}]$ is the absorptance of the rhodopsin in an unscreened photoreceptor. The MSP measured the relative extinction of a small cylindrical cross-section of the ommatidium containing screening pigment seen as an area of pure color, not pigment granules. For computational purposes, the screening pigment observed may be treated as a chemical compound. In our experimentation the test flashes were brief (0.1 s) and spaced at least 1 to 2 min apart, minimally affecting the dark-adapted state. Therefore, the threshold values for sensitivity in Figs. 1 and 3 would approximate rhodopsin in its fully dark-regenerated state in the receptors, and the effects of metarhodopsin would be almost neg-

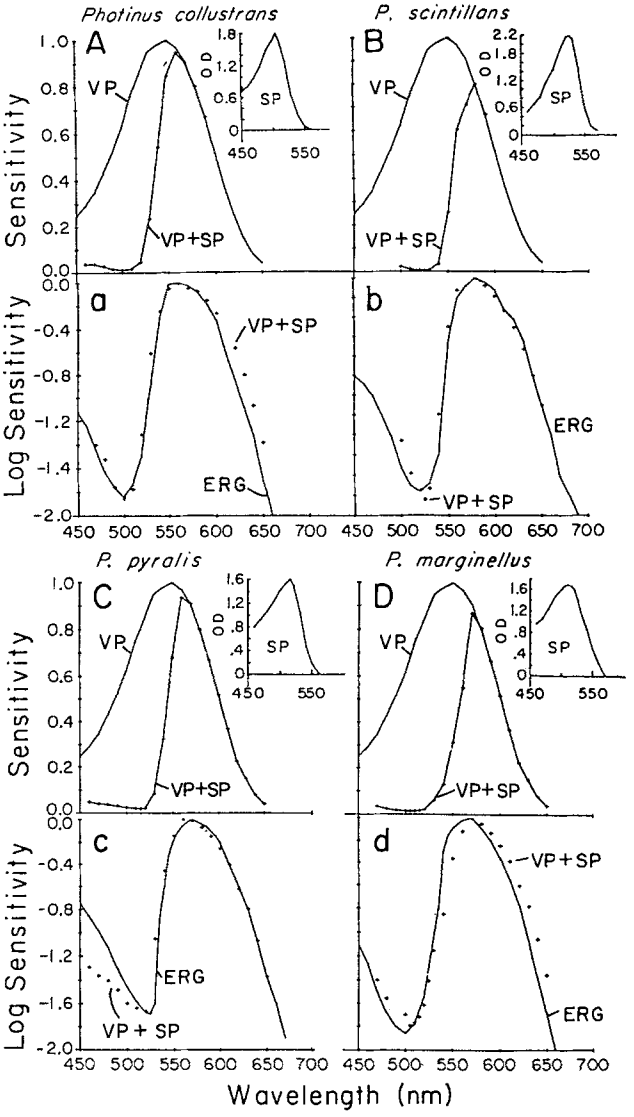

Fig. 9. Comparison of ERG $S(\lambda)$ with the calculated effect of screening pigments on the effective absorption of green photopigment (P550) as approximated by the Ebrey and Honig (1977) nomogram in: *Photinus collustrans* ♂ (**Aa**), *P. scintillans* ♂ (**Bb**), *P. pyralis* ♂ (**Cc**), and *P. marginellus* ♂ (**Dd**). Upper panels (**A, B, C, D**) show the nomogram for a P550 visual pigment (VP ●—●) and the effective modification of the assumed visual pigment (P550) by the species specific screening pigment (SP+VP, +——+) whose absorption (taken from Figs. 6 and 7) is given in the inserts (SP) assuming O.D. values from 1.6 to 2.2 in different species. The tail above 580 nm in Figs. 6 and 7 has been subtracted from the absorbance value prior to evaluation. Lower panels (**a, b, c, d**): show a comparision of dark-adapted ERG $S(\lambda)$ (——) data in Fig. 1 for each species with calculated effect (SP+VP, +) from the upper panels

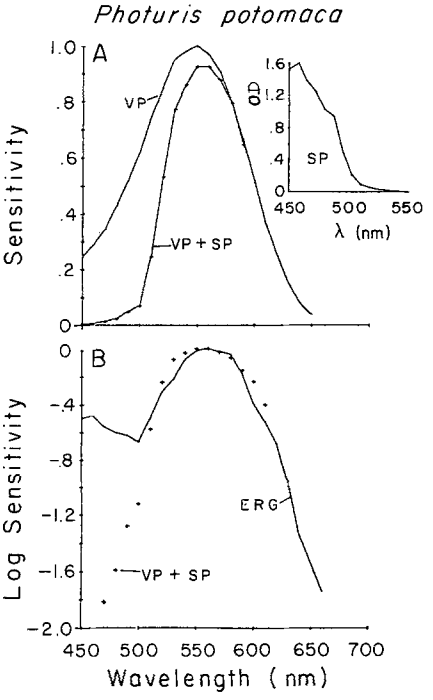

Fig. 10A, B. Comparison of ERG $S(\lambda)$ with the calculated effect of screening pigment on the effective absorption of P550 visual pigment in *Photuris potomaca* ♂. Upper panel (**A**) shows the nomogram (Ebrey and Honig 1977) for a visual pigment (VP ——) with λ_{max} at 550 nm and the modification of its effective absorption by the screening pigment (SP+VP, +——+). Insert: absorption of the screening pigment (SP) of O.D.=1.6. Lower panel (**B**) shows a comparison of dark-adapted ERG $S(\lambda)$ (——) taken from Fig. 3A with calculated effect (SP+VP, +) from the upper panel (**A**)

ligible. Hence, the Ebrey and Honig template for P550 is a good approximation for the expression $[1-10^{-D(\lambda)}]$, which can be written as $S(\lambda)$. This reduces Eq. 1 to the following expression

$$S'(\lambda)=10^{-D'(\lambda)}(S_\lambda) \qquad (2)$$

and it is plotted for different species of fireflies in Fig. 9A, B, C and D (curves labeled SP+VP). The values of $S'(\lambda)$ are then plotted on a log scale and a comparison is made between the calculated $S'(\lambda)$ and the dark-adapted $S(\lambda)$ obtained from Fig. 1A, C, E and B for *Photinus collustrans, scintillans, pyralis* and *marginellus*, and presented in Fig. 9a, b, c and d, respectively. Different OD values for screening pigment were tested, and the one plotted in the inserts of Fig. 9A, B, C and D are the values which made the best visual match between the $S(\lambda)$ in Fig. 1 and the calculated value of $S'(\lambda)$. The fit between the ERG $S(\lambda)$ and the calculated values of $S'(\lambda)$ is within one standard deviation of the ERG data points (see Fig. 1) and should be taken as evidence for the pink-to-red colored photostable pigment screens to attenuate visual sensitivity in the green in twilight-restrictive fireflies.

Similar calculations for the modification of $S(\lambda)$ were conducted for *P. potomaca* data and the results show that an overlay of 1.6 O.D. (inset Fig. 10A) of the *potomaca* yellow screening pig-

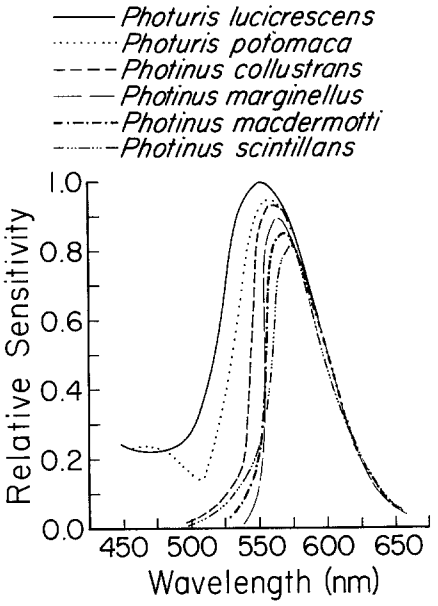

Fig. 11. Best fit of the alignment along the vertical axis of the $S(\lambda)$ functions of 4 twilight-restricted species (*P. collustrans* ♂, *marginellus* ♂, *macdermotti* ♂, and *scintillans* ♂) and one twi-night active *Photuris potomaca* ♂ with the $S(\lambda)$ function for a strictly night-active *Photuris lucicrescens* ♂. The $S(\lambda)$ functions for the twilight-active species are narrow with attenuation of sensitivity in the green and with an overall reduction in the absolute sensitivity

ment (Fig. 8A) can modify the Ebrey and Honig nomogram curve for P550 to one (Fig. 10A, curve labeled SP + VP), which corresponds with $S(\lambda)$ in Fig. 3A in the yellow-green region (Fig. 10B). Since the screening pigment in *P. potomaca* absorbs maximally in the blue, a marked attenuation of sensitivity in the blue region for $S(\lambda)$ curves was not observed (Fig. 3) and at present cannot be explained.

It should be pointed out that the colored screening pigments in the eye would reduce the overall absolute sensitivity. In Fig. 11, a comparison is made between $S(\lambda)$ of 4 twilight-restrictive species (*Photinus collustrans, marginellus, macdermotti* and *scintillans*) and one twi-night active *Photuris potomaca* with the $S(\lambda)$ of a strictly night-active *P. lucicrescens* with its absolute sensitivity taken as 100% at 550 nm. One observes that the absolute sensitivity is reduced from about 5 to 20% in among different twilight-active species. The longer the shift of λ_{max} of $S(\lambda)$ from 550 nm the more the attenuation in the absolute sensitivity.

Discussion

ERG spectral sensitivities

Narrow-yellow receptors. Lythgoe (1968) has proposed that for enhancing brightness and/or color contrast, it is necessary to have the $S(\lambda)$ offset from the maximum of the peak of the ambient illumination; it should optimally be in the lambda minimum of the ambient illumination. This indeed was shown to be true for twilight-active *P. scintillans* whose visual $S(\lambda)_{max}$ is in the yellow at 580 nm (Fig. 4b in Seliger et al. 1982b) coincided with the λ_{min} in the yellow of the skylight (Fig. 7a, in Seliger et al. 1982b) and was offset from the broad green λ_{max} around 560 nm (Figs. 7b and 8, Seliger et al. 1982b) of the sunlight reflected from green grass at twilight in the species habitat. In Figs. 1 to 3, we present data for eight species which suggest that all twilight-active fireflies possess narrow $S(\lambda)$ maxima in the yellow, and further that the sensitivity in the green is markedly reduced in these species which restrict their flashing activity to a short period at twilight (Fig. 1). Besides the peak sensitivity in the long wavelengths, a secondary peak is always present in the short wavelength region (Figs. 1, 2, 3). The two peaks, yellow and blue-violet were isolated by appropriate selective adaptation experiments (Fig. 2). Recently Eguchi et al. (1984) have shown that Japanese fireflies also possess long as well as short wavelength sensitivity similar to that indicated by our data.

Short wavelength receptors. The presence of structurally specialized ommatidia in the dorsal rim of the compound eyes is common among insects (Apeli et al. 1985; Meinecke 1981; Wunderer and Smola 1982). The ommatidia are specialized to analyze the polarized light of the sky with UV (honeybee; Labhart 1980) and blue (cricket; Labhart et al. 1984) receptors. The dominant sensitivity in the near-UV (*P. scintillans*, Fig. 2) and blue (*P. frontalis*, Fig. 4) would favor the interpretation that the dorsal retina in the firefly may also be specialized.

Based on the data presented in Figs. 1, 2 and 3, the presence of three receptor types: UV, blue and yellow, in the compound eyes of twilight-active fireflies is strongly suggested, which is similar to what was suggested earlier (Lall et al. 1980a, 1982) for dark-active fireflies. However, intracellular optical physiological data are essential for confirmation of the above hypothesis.

Screening pigments

The absorption spectra of pigment granules present in the distal sleeve of the firefly eyes (Fig. 5) are comparable to the ones found among other insects (review: Langer 1975; Höglund et al. 1970) and Crustacea (Stowe 1980). These pigments essen-

tially function as neutral density filters leaky at long wavelengths. The attenuation presumably is about 2.3 units (Höglund 1966).

The physiological mechanism for modifying $S(\lambda)$ by screening pigment(s), specifically the oil droplets, is of common occurrence in the avian (Bowmaker 1979; Sillman et al. 1981; Goldsmith et al. 1984) and the turtle (Granda and Haden 1970; Lipetz 1984) retinas. It was proposed that vision in fireflies in the visible region is due to a green absorbing visual pigment (P550) which could be modified by screening pigment(s) (Fig. 2; Seliger et al. 1982a). Such a screening pigment was reported in *P. pyralis* (see Fig. 11 in Seliger et al. 1982b) and is now presented for 6 more species (Figs. 6, 7, 8). The absorption spectra of these colored screening pigments in the eyes of fireflies of genus *Photinus* (Figs. 6 and 7) appear to be uniquely different from the shapes of the absorption spectra of other screening pigments thus far described in invertebrates (Langer 1975; Goldsmith 1978; Stowe 1980; Strother and Casella 1972; Strother and Superdock 1972), and undoubtedly is the most intriguing aspect of our findings. These are relatively narrow band, sharp cut-off filters with maximal absorption only in the green region of the spectrum, allowing both the short and particularly the long wavelength sensitivity to remain almost unaltered. Since rhodopsins possess relatively broad-band absorption characteristics, it would be difficult to explain a sharp decrease in green wavelength sensitivity by any other mechanism like wavelength-specific light absorption by the cornea or by retinal lateral inhibition. The presence of the screening filters in Figs. 6 and 7 would narrow the broad green $S(\lambda)$ due to P550 rhodopsin alone, with the maximum shifted toward longer wavelength as observed in Fig. 1. Furthermore, the matching of our ERG $S(\lambda)$ curves (Fig. 1) with the value of $S'(\lambda)$ in Eq. 2 suggests that the density of the photostable colored screening pigment ranges in 1.6–2.2 optical density units among different species of fireflies (Fig. 9).

Screening pigments, contrast enhancement and mesopic vision

At moderate and low intensities, in mesopic range of illumination, the amount of information obtained from any image is a function of the number of photons captured by the photoreceptors (Lythgoe 1984). In accordance with Poisson statistics, the larger the sample counted, the finer the differences between samples that can be detected. There are two ways for improving the detection of the difference between the two elements of the retinal image: a) by increasing the number of photons sampled (increasing the brightness) or b) by increasing the relative difference between the number of photons in each image element (increasing the contrast). Based on the Poisson distribution, Land (1981) estimated that to make a contrast discrimination at 95% level, a 100-fold decrease in brightness can be compensated for by a 10-fold increase in contrast. At the photoreceptor level, contrast can be improved by making the spectral sensitivity maximum at wavelengths where the relative photon reception between the object and its background is greatest (Lythgoe 1984). The narrower $S(\lambda)$ curve of the photoreceptor, the more precisely it can be tuned to increase contrast. This narrowing of $S(\lambda)$ in fireflies (Figs. 1, 3) has now been shown to be accomplished by colored screening pigments (Figs. 6, 7, 8) and confirms our earlier hypothesis (Lall 1981b) that, indeed, colored pigments attenuate visual sensitivity in the green. Red screening pigments in the rhabdomeric region have been reported in butterflies (Ribi 1979) and in digger wasp (Ribi 1978), and have been implicated for enhancement of color contrast against a background of green foliage field, over which these insects fly.

A correlation exists between the color of the screening pigment and the activity period among twilight-restrictive species. A red screening pigment (λ_{max} 525 nm) was found in *P. scintillans*, which initiates flashing early at twilight, while pink pigment (λ_{max} 512 nm) exists in *P. collustrans* which initiate flashing late at twilight, and a magenta pigment in *P. pyralis* (λ_{max} 517 nm) and *marginellus* (λ_{max} 512 nm) species, which initiate flashing at mid-twilight. Hence it is suggested that a red screening filter is needed to reduce 'noise' at early twilight when the intensity of the green-reflected light is about 1 log unit more than 20 min later at twilight (see Fig. 6 in Seliger et al. 1982b). The differences in the peaks of the screening pigments in Figs. 6 and 7 could then account for the presumed differences in the characteristics of the sunlight reflected from green foliage in different habitats and at different times during twilight. However the presence of these colored pigments reduces (5–20%) the absolute sensitivity of the visual system as summarized in Fig. 11.

The broad absorption in the blue of the yellow screening pigment in *Photuris potomaca* and *versicolor* (Fig. 8) differs dramatically from the colored pigments in genus *Photinus* (Figs. 6, 7). *P. potomaca* yellow screening pigment (λ_{max} 461 nm) has a small effect on P550 mediated vision in the green of $S(\lambda)$ (Figs. 3A, 10), whereas *P. versicolor* yellow

screening pigment has no effect in the green (Lall 1981a). Since *P. potomaca* flashing activity extends from twilight into night, and *P. versicolor* is a night-active species, the problem of 'noise' in the dark is not encountered in the latter species. Therefore, these yellow pigments in night-active fireflies have a distinctly different role in vision than the pink-to-red screening pigment found in twilight-restricted fireflies.

In summary, it is intriguing to report the presence of a series of unusual screening pigments with narrow absorption in the green which filter light selectively and modify broad green sensitivity into narrow yellow visual spectral sensitivity in twilight-restrictive fireflies. These screening filters form a series of cut-off filters, graded to the photon intensity of the 'noise' (sunlight reflected from vegetation) at twilight and enhancing brightness and/or color contrast and reducing glare, in order to optimize the detection of bioluminescent signals in twilight-active fireflies.

Acknowledgments. We have had valuable discussions with Professor James E. Lloyd, and his contributions to the firefly project are gratefully acknowledged. William H. Biggley has been an enthusiastic consultant both in theoretical discussions and in laboratory techniques, Phillip Chapados provided excellent technical support in electrophysiology and Dorothy Regula provided unfailing support in preparation of this manuscript. Dr. J. Cicero generously provided specimens for *Bicellonycha wickershamorum* from Arizona. Supported by NSF BNS-8311567 and BNS-8518769 (to TWC), BNS-8311127 (to H.H.S.), NIH grant #5 RO1 EY 00520 (to Richard A. Cone) and a grant from Mr. Robert L. Conway (to A.B.L.).

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