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Ultraviolet Photoreception in Mantis Shrimp

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An UV-sensitive class of photoreceptors exists in all regions of the retinas of mantis shrimps. UV photosensitivity apparently resides in rhabdomeres of the eighth retinular cell (R8) that lies atop each rhabdom; and in ommatidia where the R8 rhabdomere consists of microvilli parallel in a single direction, sensitivity is maximal when the e-vector of plane-polarized light is parallel to the microvilli. Spectral sensitivity of the UV photoreceptor peaks at 345 nm and is best explained by the presence of a photopigment with λ_{max} near 325 nm overlain by material that absorbs UV light at wavelengths below $\approx$ 350 nm. Rhabdomeres of R8 cells in several different retinal regions of a variety of species examined contain a photopigment absorbing maximally below 340 nm. Under appropriate conditions, a metapigment with λ_{max} near 460 nm can be formed. UV vision may be useful for enhancing the visual contrast of midwater predators or prey.

Ultraviolet Compound eye Stomatopod Crustacea Polarization sensitivity

INTRODUCTION

While only recently has it become clear that UV vision occurs in a variety of vertebrates (see review of Jacobs, 1992), it has been known for a long time that many invertebrates—particularly arthropods—are sensitive to UV light (review Menzel, 1979). However, the great majority of species known to have UV vision are terrestrial. Relatively few aquatic invertebrates have had their visual systems examined for UV photosensitivity, but the results that do exist suggest that short-wavelength photoreceptor classes are rare in such species. When present, these receptors tend to be maximally sensitive to near-UV or short-visible spectral regions. For example, microspectrophotometric and electrophysiological measurements have revealed receptor classes with peak sensitivity at about 440 nm in crayfish (Goldsmith & Fernandez, 1968; Cummins & Goldsmith, 1981), aquatic crabs (Martin & Mote, 1982) and one species of land crab (Lall & Cronin, 1987).

It is commonly assumed that there would be no reason for aquatic animals to have short-UV photosensitivity, since natural waters transmit only limited quantities of UV radiation. Marine waters in particular are often thought to be virtually devoid of UV. In actuality, natural waters can be quite transparent to UV light; the clear waters of the open ocean may have relatively small extinction coefficients at 300 nm or even shorter (Smith & Baker, 1979). It should be no surprise, then, to learn that some aquatic species possess true UV photosensitivity. Among these are the freshwater cladoceran, *Daphnia magna*, which has a 350-nm receptor class (Smith & Macagno, 1990), the spiny lobster, *Panulirus argus*, with a 370-nm receptor class (Cummins, Chen & Goldsmith, 1984), as well as several species of deep-sea crustaceans that have receptor classes of λ_{max} between 350 and 400 nm (Frank & Case, 1988). Besides these crustacean species, the marine xiphosuran *Limulus polyphemus* has a class of photoreceptors in its median eye that are maximally sensitive at 360 nm (Wald & Krainin, 1963; Chapman & Lall, 1967; Nolte & Brown, 1970).

The visual systems of stomatopod crustaceans (mantis shrimps) are among the most complex known. Each compound eye has three distinct regions: the dorsal and ventral hemispheres, which have extended fields of view, separated by a midband (usually containing six parallel rows of ommatidia, numbered from Row 1 to Row 6, dorsally to ventrally) viewing only a narrow strip of space (Exner, 1891; Demoll, 1909; Horridge, 1978; Marshall, 1988). Ommatidia of the midband are specialized for a complex analysis of the spectrum and polarization of light (Cronin & Marshall, 1989a, b; Cronin, Marshall & Caldwell, 1993; Marshall, Land, King & Cronin, 1991a, b). Many stomatopod species have 10 classes of photoreceptors, with peak sensitivities at wavelengths from 400 to beyond 600 nm.

Mantis shrimps are also known to have UV photosensitivity (Schiff, 1963; Cronin, 1989). In the work reported here, we set out to examine the properties of the UV photoreceptors of mantis shrimps, including their spectral sensitivity, their distribution throughout the retina and their cellular identity within the retina. Our results show that several species of stomatopod crustaceans have UV photoreceptors which occur in all classes of ommatidia of the retina and which have unusual spectral and polarizational properties.

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MATERIALS AND METHODS

Animals

We examined several stomatopod species in this study, all members of superfamilies Gonodactyloidea and Lysiosquilloidea. Specimens were either collected by us for shipment to the laboratory or were supplied by professional collectors. Animals were maintained in marine aquaria and were typically used for experiments soon after their arrival at the laboratory.

Intracellular optical physiology

All experiments of this type involved the gonodactyloid species *Gonodactylus oerstedii*. The techniques used were developed by Franceschini (1975) and further refined by Bernard and Stavenga (1979). Our applications have been described in detail by Cronin (1989) and by Cronin and King (1989). Briefly, an animal was attached by the dorsal surface of the carapace to an adjustable, submersible stage. Quick-setting epimine dental plastic was used for this and was also employed to immobilize the eyes. After being placed in artificial seawater, the stage and animal were adjusted so that the three deep pseudopupils (DPPs) of one of the eyes faced upwards (see Cronin, 1989) and the animal was placed in the dark for several hours.

Following dark adaptation, the image of the DPP to be examined (containing about 10 ommatidia in the dorsal or ventral hemisphere of the eye and as few as three ommatidia in rows of the midband) was centered in the field of view of a Nikon incident-light microscope, using a Zeiss 25-mm Luminar objective that had previously been demonstrated to transmit UV light to wavelengths as short as 330 nm. The objective was enclosed in a diptube having an angled coverslip as a window (see Cronin, 1989). The DPP was illuminated with far-red light (wavelengths > 780 nm, Schott RG800 or Ditric 780 nm long-pass filter) and the portion of this measuring beam that was scattered back from the DPP was sent to a photometric head (Zeiss 01K, containing a Hamamatsu R928 photomultiplier tube) for measurement via a PARC 1140 quantum photometer connected to an A/D interface in a microcomputer. To prevent contamination of the measuring beam with the stimulating beam, the light passed through a 720-nm long-pass filter before entering the photometric head.

The stimulating beam was brought onto the eye using the epi-illumination arm of the microscope, on the same optical axis as the measuring beam, and was controlled by the microcomputer using a Uniblitz electromagnetic shutter, an Oriel grating monochromator and counterrotating quartz circular density wedges (density range, 0–3.5 O.D.). Stimulation was provided by a 150-W or 75-W Xenon arc; a Schott UG1 UV-transmitting filter was used for order separation and a sheet of Polaroid HNP'B polarizer was used to control the axis of polarization. The quantal irradiance at each test wavelength and polarization was measured prior to each experiment using a calibrated United Detector Technologies PIN-10DP/SB photodiode.

To examine the intracellular optical physiological response to stimulation with UV light, the eye was allowed to dark adapt for 2 min between stimulations. A stimulus (typically 360 nm in wavelength, lasting 30 sec) was provided and the change in reflectance of the measuring beam during stimulation was recorded (sampling frequency, 1 kHz), as well as the subsequent return to baseline reflectance following the stimulus.

Measurements of spectral sensitivity of the UV photoreceptor class were made using the "scan technique" (Cronin & King, 1989). In this technique, the intensity of the stimulating light is varied at each wavelength to "light-clamp" the reflectance from the DPP at a preselected level. The advantage of this approach is that an accurate measurement of spectral sensitivity can be completed within a few minutes. See Cronin and King (1989) for technical details.

Microspectrophotometry

Individuals were dark adapted overnight or longer, after which eyes were removed in the dark, briefly fixed (2–4 hr) in a solution of 2.5% glutaraldehyde in pH 7.5 marine crustacean Ringer's solution (Cavanaugh, 1956), quick-frozen using fluorocarbon ("Freon") spray and mounted in a cryostat. Sections were cut under dim yellow light (containing no UV) at thicknesses from 10 to 14 μm, mounted within a ring of silicon grease between coverslips in either mineral oil or in the same glutaraldehyde solution as that used to fix the eye, and placed in the microspectrophotometer (MSP).

The instrument used was a single-beam MSP; our techniques have been described previously (Cronin & Forward, 1988; Cronin & Marshall, 1989a, b; Cronin *et al.*, 1993) and only the special requirements of UV microspectrophotometry will be discussed in detail here. Sections of rhabdomeres to be scanned were positioned while illuminated by light that had wavelengths below 400 nm removed by an Oriel 5147 400-nm long-pass filter and scanned using a circular beam 2.5 μm in diameter. Scans were made from 300 to 600 nm, with data recorded at 1-nm intervals. However, the objectives used in the MSP (Nikon UV-F, 100X) transmitted measurable quantities of UV light only at wavelengths beyond 325–330 nm, so the effective range of the spectral scans was 330–600 nm. Before scanning a photoreceptor, the beam of the MSP was placed in a clear area of the preparation for a reference scan; the reference scan was stored for later computation of the density of the scanned photoreceptor at each wavelength.

Actinic treatments of the photoreceptor were provided by illuminating the preparation brightly with light from either the microscope's substage illuminator or a 75-W Xenon arc, passed through an UV-transmitting filter (Schott UG1) to bleach the UV photopigment (in glutaraldehyde medium) or to photoconvert it to metapigment (in mineral oil medium). Photoregeneration of the photopigment from the metapigment (in mineral oil) was performed by passing the beam of the substage illuminator through an Oriel 5147 filter; such a beam contained only wavelengths longer than 400 nm.

Shapes of difference spectra generated either by photoconverting between the photopigment and the metapigment, or by photobleaching the photopigment, were compared with template spectra for rhodopsin or metarhodopsin supplied by G.D. Bernard (see Bernard, 1987; Cronin & Marshall, 1989b). The template was derived from data for mid-wavelength visual pigments (λ_{max} near 500 nm) and may not be fully applicable to visual pigments absorbing maximally in the UV. At present, however, no template spectra have been derived for UV-rhodopsins and the Bernard function proved to be adequate for our purposes.

Underwater UV and visible-light photography

To take photographs underwater, we used a Nikonos III camera equipped with Kodak Tri-X film (ASA 2000 with extended processing). Photographs were taken through a glass 35-mm lens (effective focal length underwater, 50 mm). To take photographs in the UV, a Corning 7-54 filter (> 50% transmission from 265 to 385 nm, peak transmission at 325 nm) was placed in front of the lens. The combination of the glass lens with the filter reduced the spectral range in the photographs to about 350–390 nm and required an exposure increase of 5–6 stops over the exposure without the filter.

RESULTS

Pupillary responses of UV photoreceptors

The intracellular optical technique measures changes in reflectance from within the deep pseudopupil of a compound eye as the individual photoreceptor classes adapt to photic stimulation (Franceschini, 1975; Stavenga, 1979; Bernard & Stavenga, 1979; Cronin, 1989). Ommatidia in the peripheral regions of compound eyes of *G. oerstedii* respond differently to stimulation by visible or by UV light [Fig. 1(A)]. In response to light with a wavelength of 500 nm, a rapid rise in pupillary reflectance is observed, reaching a steady state within 10–15 sec. Following stimulation, reflectance returns to the dark-adapted level rapidly, in less than 10 sec [Fig. 1(A), thin line]. Responses of identical steady-state amplitude upon stimulation with UV light (wavelength of 360 nm) are much slower, reaching a plateau in about 30 sec and not returning fully to the dark-adapted state even after 30 sec in the dark [Fig. 1(A), thick line]. In both cases the response level is below the level of saturation and the fact that the time–courses to the visible and UV stimuli differ suggests that at least two classes of photoreceptor are present, responding maximally to visible and to UV light (see also Cronin, 1989).

Photoreceptors sensitive to UV light are present in all types of ommatidia in the triple compound eye of *G. oerstedii*, being found in both hemispheres and in all six rows of the midband [Fig. 1(B)]. When stimulated with identical intensities of UV light, all ocular regions express pupillary responses. Although the magnitude of the response varies among these regions, the time–courses of all responses reach steady-state levels of reflectance in 15–30 sec and return slowly to baseline levels on completion of stimulation [Fig. 1(B)]. In contrast, stimuli at longer wavelengths produce a variety of response forms (see Cronin, 1989).

Cellular identity of the UV photoreceptor class

We hypothesized that UV photosensitivity in mantis shrimp compound eyes resides in the eighth retinular cell (R8). This cell type is present at the top of the rhabdom in all ommatidia (Marshall *et al.*, 1991a) and contains no visual pigments that absorb light at wavelengths beyond 400 nm (Cronin & Marshall, 1989b). Furthermore, R8 is known to be a short-wavelength (though not necessarily UV) receptor class in other crustacean species (Cummins & Goldsmith, 1981; Martin & Mote, 1982; Cummins *et al.*, 1984). Unlike the R8s of most crustacea, which are devoid of screening pigment and therefore incapable of producing an intracellular optical response, R8 cells of stomatopods contain abundant granules of screening pigment near the rhabdom (see Figs 23–27 in Marshall *et al.*, 1991a). Stomatopod R8 cells might, therefore, exhibit changes in reflectance upon light adaptation.

We were able to test directly the hypothesis that R8 cells respond to UV light by exploiting an unusual feature of stomatopod retinas. In species like *G. oerstedii* that have six-row midbands, the rhabdomeres of the R8

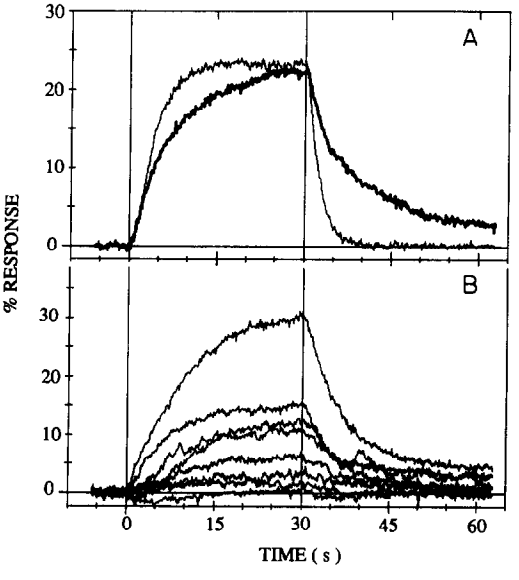

FIGURE 1. Pupillary responses, as percent reflectance change compared to background levels of reflectance (% response), obtained from the deep pseudopupils of compound eyes of the stomatopod species *Gonodactylus oerstedii*. In both panels, stimulation commenced at the first vertical line (0 sec) and ended at the second vertical line (30 sec). (A) Responses from ommatidia in the dorsal hemisphere of the eye. Intensities were chosen to match final response levels, below the level of saturation, to stimulation at 500 nm (4.23×10^{11} photons cm^{-2} sec^{-1}, thin trace) and 360 nm (3.28×10^{12} photons cm^{-2} sec^{-1}, bold trace). Each trace represents the average of three separate responses. (B) Responses to stimulation with UV light (360 nm, 3.86×10^{12} photons cm^{-2} sec^{-1}) in both hemispheres and all six rows of the midband. From least to greatest, plateau phases indicate responses originating from Row 1, Row 2, Row 3, Row 6, Row 4, Row 5, the ventral hemisphere and the dorsal hemisphere. Each trace is a single response.

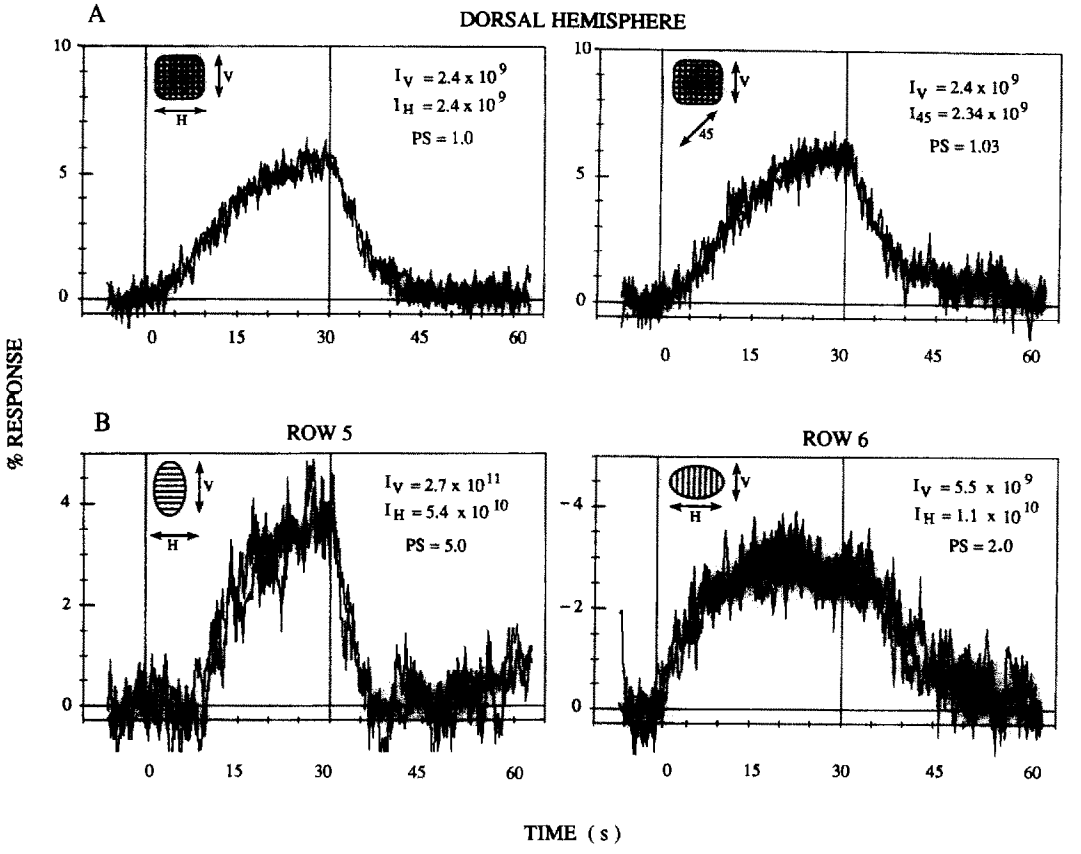

FIGURE 2. Polarization sensitivity (PS) of pupillary responses of ommatidia in the dorsal hemisphere and in midband Rows 5 and 6, in compound eyes of *Gonodactylus oerstedii*. Responses are plotted as in Fig. 1 and each panel shows two matched responses. The inset figure in the top left part of each panel suggests the cross-sectional shape of the rhabdomeres of R8 cells in that part of the retina, with the microvillar directions illustrated by cross-hatching (dorsal hemisphere) or parallel lines (Rows 5 and 6). The two-headed arrows show the orientations of the e-vectors of the polarized-light stimuli; H, horizontal; V, vertical; 45, at 45 deg. Intensities of stimuli (photons cm^{-2} sec^{-1}) are given in the upper right corner of each panel (I_V, intensity for vertically polarized stimulus; I_H, intensity for horizontally polarized stimulus; I_{45}, intensity for stimulus at 45 deg). The ratio between the intensities required for matched responses is the polarization sensitivity (PS), which is also given in the upper right part of each panel. (A) Matched responses from the deep pseudopupil of ommatidia in the dorsal hemisphere, where the rhabdomeres of R8 cells have mutually perpendicular microvilli. PS is near 1.0 for all orientations of polarized light. (B) Responses from deep pseudopupils in ommatidia of Rows 5 (left) and 6 (right) of the midband. Rhabdomeres of R8 cells in these retinal regions have unidirectional, parallel microvilli; but the microvilli or Row 5 are oriented perpendicular to those of Row 6. PS is substantially greater than 1 for ommatidia in these retinal regions. Note that the responses from Row 6 were in the negative direction in this case and have been inverted for plotting.

cells of the two most ventral rows of the midband (Rows 5 and 6) have unidirectional microvilli. Furthermore, the directions of the microvilli in the two rows are mutually orthogonal; microvilli in R8 cells of Row 5 extend parallel to the equator of the eye, while those of Row 6 are perpendicular to the plane of the equator (Marshall, 1988; Marshall *et al.*, 1991a). If UV-absorbing visual pigments are like other visual pigments and have chromophores that lie parallel to the axes of the microvilli, these photoreceptors with unidirectional microvilli should vary in sensitivity according to the plane of the e-vector of polarized light. Specifically, UV photoreceptors of Row 5 ommatidia should be maximally sensitive to plane-polarized light having its e-vector parallel to the equator of the eye, while ommatidia in Row 6 should be more sensitive to the orthogonal plane of polarization of light.

We therefore tested polarization sensitivity (PS) to plane-polarized UV light in photoreceptors of Rows 5 and 6. We used photoreceptors of the dorsal hemisphere as controls, since the rhabdomeres of R8 cells in this region have microvilli that are mutually perpendicular in the same cell (Marshall *et al.*, 1991a) and should have equal sensitivity to any plane of polarization of axially incident UV light. In our experiments, horizontal e-vector orientations were parallel to the eye's equator. Therefore, UV-sensitive cells of Row 5 were expected to be more sensitive to the horizontal orientation of the e-vector and those of Row 6 to the vertical orientation. In contrast, if there is no induction of polarization by the optics of our apparatus, UV-sensitive cells in the dorsal hemisphere should respond identically to all planes of polarization. We determined relative sensitivity to varying planes of e-vector orientation by stimulating the eye with appropriate intensities of light ($\lambda = 360$ nm) to produce matched responses at each e-vector orientation.

Typical results are illustrated in Fig. 2. Figure 2(A) shows matched responses of UV-sensitive photoreceptors in the dorsal hemisphere to light polarized vertically, horizontally and at 45 deg. Sensitivity was

identical at all tested e-vector orientations, showing that PS = 1.0 as expected. However, UV polarization sensitivity exceeded 1.0 in rows where R8 cells have unidirectional microvilli [Fig. 2(B)]. In Row 5, sensitivity was greater to horizontally polarized light, while in Row 6, the greater sensitivity was to light of vertical polarization. Not only do these results correspond with prediction, but the observation that optimal e-vector orientations for Rows 5 and 6 are mutually orthogonal demonstrates that the results are not due to an artifactual variation in polarization produced by the apparatus. In nine measurements of PS in Rows 5 and 6, PS ranged from 1.94 to 5.05 (mean PS of 3.01 ± 1.06 SD), with the greater sensitivity always in the predicted direction. We therefore conclude that the R8 cells are UV photoreceptors in *G. oerstedii.*

Spectral sensitivity of the UV photoreceptor

The spectral scanning technique developed by Cronin and King (1989) was used to measure spectral sensitivity in the UV. Since ommatidia of the dorsal hemisphere tended to produce the largest reflectance changes upon UV stimulation [Fig. 1(B)], UV sensitivity was measured in this region of the compound eye. Sensitivity reached its maximum at 345 nm, declining rapidly on both sides of the peak [Fig. 3(A)]. When compared to standard visual pigment template spectra (Bernard, 1987) having λ_{max} between 320 and 350 nm, the sensitivity spectrum is very narrow, falling particularly rapidly on the long-wavelength limb [Fig. 3(A)]. However, when wavelengths below 350 nm are omitted and the remaining data are rescaled by eye for an optimum fit, the long-wavelength limb of the sensitivity spectrum provides a good approximation of the template spectrum, assuming a visual pigment λ_{max} near 325 nm [Fig. 3(B)]. It seems very likely, therefore, that the reduction in sensitivity at shorter wavelengths is due to absorption of UV light by the cornea and crystalline cones, whose absorbance increases rapidly as wavelengths fall below 360 nm (Cronin & Marshall, 1989b).

UV microspectrophotometry of rhabdomeres of R8 cells

We searched for UV-absorbing visual pigments in rhabdomeres of R8 cells using microspectrophotometry. It became clear very early in this phase of the research that the UV photopigments present in the R8 rhabdomeres were very unstable, bleaching particularly rapidly in the presence of glutaraldehyde fixative (2.5% in marine crustacean Ringer's) or even in the presence of the Ringer's solution without the addition of glutaraldehyde. However, when the sectioned material was suspended in mineral oil (originally used in an attempt to reduce scattering at very short wavelengths), both the native photopigment and its photoproduct were thermally stable and, on occasion, capable of numerous interconversions.

The results of such a series of photoconversions are portrayed in Fig. 4. In this case, the data were obtained from the R8 rhabdomere of an ommatidium from midband Row 1 of *Lysiosquilla sulcata.* Figure 4(A) shows difference spectra for photoconversions between the UV photopigment and its photoproduct, produced by actinic phototreatment. The results were produced by providing the photoreceptor with five pairs of exposures, first to UV light (30 sec of exposure using a UG1 filter with bright tungsten light) and then to light with the UV

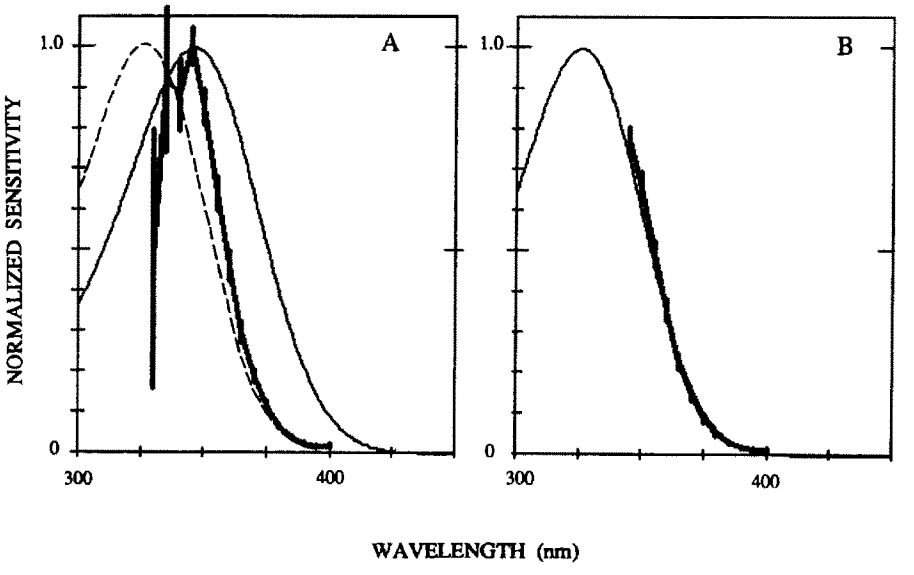

FIGURE 3. Spectral sensitivity of pupillary responses in ommatidia of the dorsal hemisphere of compound eyes of the stomatopod species *Gonodactylus oerstedii.* (A) Spectral sensitivity from 330 to 400 nm, obtained using the "scan" technique [bold solid trace (see Cronin & King, 1989)]. Data are plotted at 5-nm intervals (± SEM). The data were obtained from 21 separate scans, in 12 experiments. Spectral bandwith is very narrow and the data could not be fit to any visual pigment template spectrum. For comparison, template spectra from rhodopsins of λ_{max} = 325 nm (dashed trace) and λ_{max} = 345 nm (thin solid trace) are included. (B) Same data as in (A), but illustrating only the results beyond 350 nm (bold trace). By rescaling, this region of the sensitivity spectrum can be fit by eye to a template spectrum for a visual pigment with λ_{max} = 325 nm (thin trace).

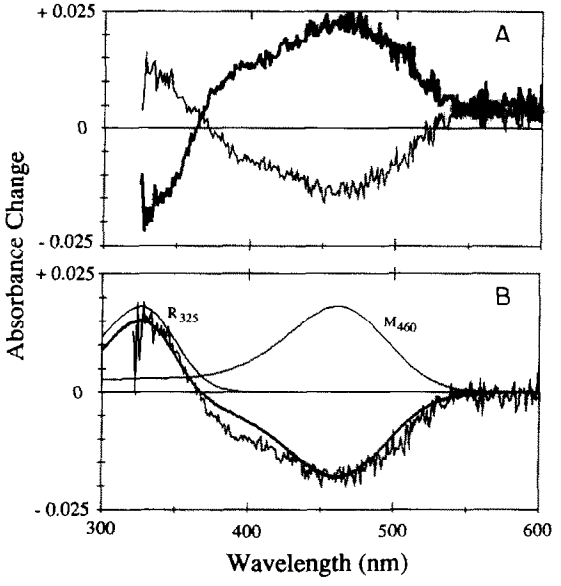

FIGURE 4. Difference spectra for photoconversion obtained from the rhabdomere of an R8 cell in Row 1 of the midband of a compound eye of *Lysiosquilla sulcata*. (A) Averages of five photoconversions produced by treatment with UV light (bold trace) and light containing no wavelengths below 400 nm (thin trace). See text for details. (B) Average difference spectrum for photoconversion (jagged trace) for all treatments of (A), following inversion of the data produced by UV phototreatment. The experimentally derived difference spectrum was matched by eye to a synthetic difference spectrum (bold smooth trace) for photoconversion between a rhodopsin with λ_{max} of 325 nm (R_{325}) and a metarhodopsin with λ_{max} of 460 nm (M_{460}) (thin smooth traces), with equal molar extinction at the peaks of the rhodopsin and metarhodopsin spectra.

component removed (30 sec of exposure using a 400-nm long-pass filter with the same bright tungsten light; see Methods). The absorption spectrum of the receptor was measured at the beginning of the series of phototreatments (when still dark adapted) and after each actinic exposure; difference spectra were computed for the effects of each exposure. The dark trace shows the average result for five photoconversions with UV-light exposure, which produced a loss of density at wavelengths below ≈ 375 nm and a density gain from ≈ 375 to ≈ 550 nm, peaking at about 460 nm. The alternating five treatments with light containing no UV component produced an average curve of the opposite shape (light trace). The symmetry of the two curves provides strong evidence for a two-component photosensitive system, including a photopigment absorbing maximally at short-UV wavelengths that interconverts with a photoproduct absorbing at short, visible wavelengths.

The spectral locations of the photopigment and its product were estimated by averaging all 10 photoconversions (inverting the ones produced by treatment with UV light) and fitting template spectra by eye to the resulting difference spectrum [Fig. 4(B)]. The data are very well fit by a difference spectrum produced by the interconversion of a rhodopsin of $\lambda_{max} = 325$ nm with a metarhodopsin with $\lambda_{max} = 460$ nm [Fig. 4(B), bold smooth trace].

Further evidence for the presence of a photopigment in R8 rhabdomeres that absorbs at unusually short wavelengths was provided by photobleaching the pigment, using cryosections mounted in the glutaraldehyde medium (Fig. 5). To obtain difference spectra for photobleaching, the photoreceptors were first scanned while dark adapted and subsequently after 15 or 30 sec of exposure to a bright UV beam produced by passing light from a 75-W Xenon arc through a UG1 UV-transmitting filter. Although many photoreceptors produced no useful data, all species examined had R8 cells that contained a photosensitive pigment bleaching maximally at wavelengths between 325 and 350 nm. Examples in Fig. 5 are averages from two R8 photoreceptors in Row 5 of the gonodactyloid species *Hemisquilla ensigera* [Fig. 5(A)] and from three R8 photoreceptors in Rows 5 and 6 of the lysiosquilloid *Lysiosquilla maculata*. Similar data, of generally lower quality, were obtained from three other gonodactyloid species: *Pseudosquilla ciliata*, *Pseudosquilla n. sp.* and *G. oerstedii*. Data suggesting the presence of visual pigments with peak absorption in the UV were obtained from a variety of R8 rhabdoms, both in the midband and in the periphery.

DISCUSSION

In this project, we investigated UV photoreception in mantis shrimps both physiologically in the intact compound eye and microspectrophotometrically in single

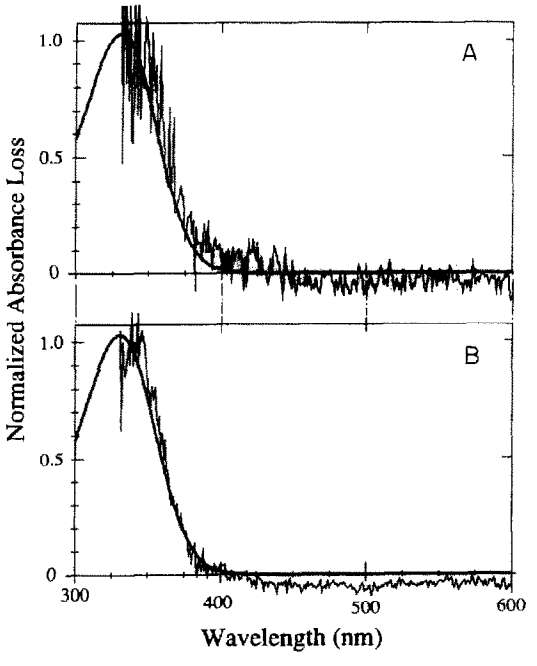

FIGURE 5. Average difference spectra for photobleaching of visual pigments in rhabdomeres of R8 cells in two species of stomatopods. In both panels, the data are represented by the jagged traces and a template spectrum for a rhodopsin with λ_{max} at 330 nm is plotted as the bold smooth curve. (A) *Hemisquilla ensigera*: average from two photoreceptors in Row 5 of the midband. (B) *Lysiosquilla maculata*: average from three photoreceptors in Rows 5 and 6 of the midband.

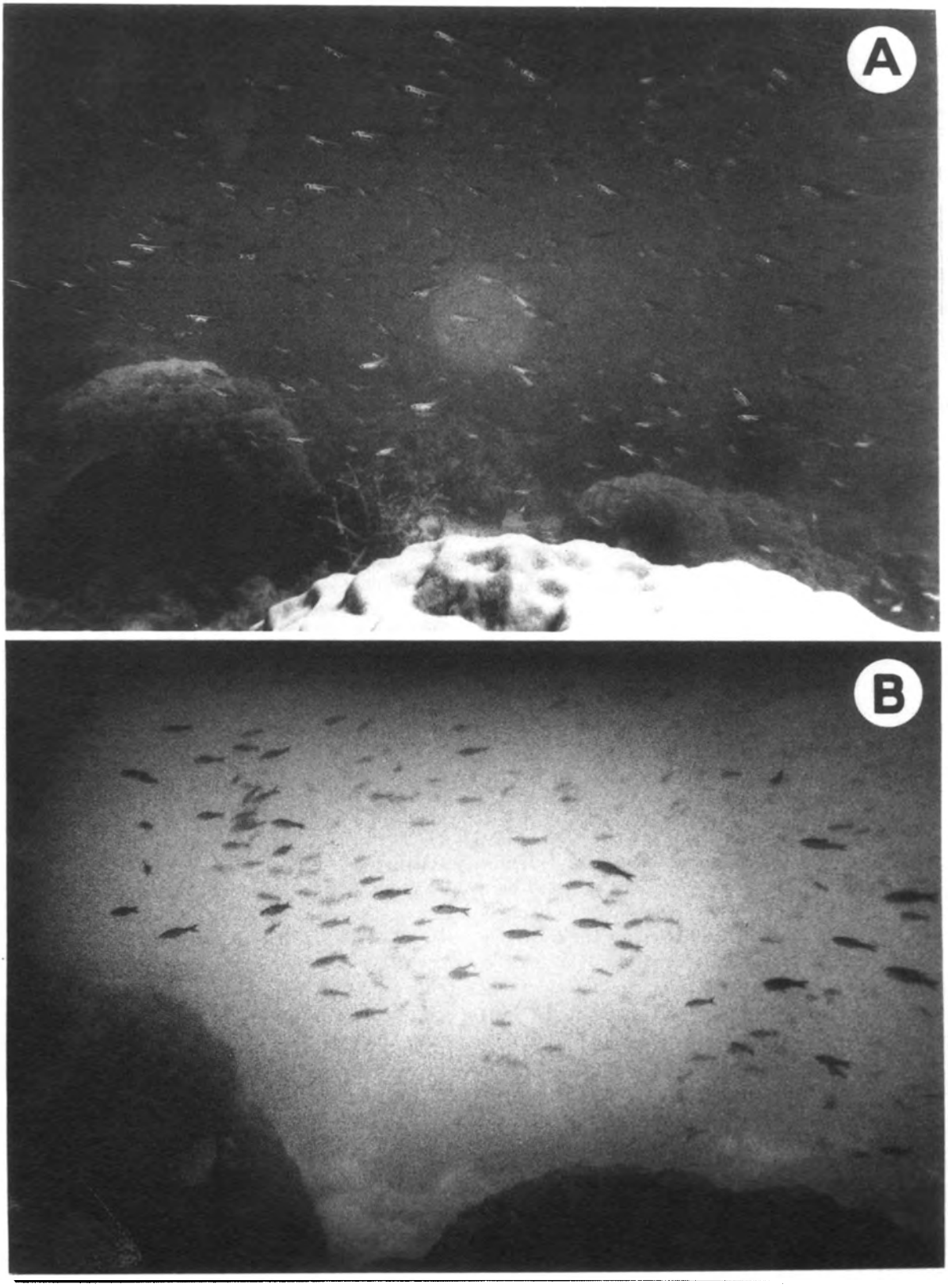

FIGURE 6. Images of a school of damsel fish over coral near Lizard Island, Australia, taken in "visible" light (A) and UV light (B). See Methods section for a description of our technique. Note the striking contrast of the fish against the strongly scattered UV spacelight, making them much easier to distinguish in the UV than in "visible" light.

photoreceptors. Results of both techniques are in excellent accord and together suggest the presence of a photoreceptor class containing an UV photopigment of unusually short λ_{max}. A variety of special properties of the UV receptor class will be discussed here.

Pupillary responses to UV light

UV photosensitivity resides in all classes of ommatidia throughout the eye of *G. oerstedii* [Fig. 1(B)]. Pupillary responses to UV stimulation are relatively slow

compared to those evoked by visible-light stimulation. Similarly sluggish responses have been seen in UV receptors of bees (Bernard & Wehner, 1980) and butterflies (Bernard, personal communication). The different types of responses to visible and UV light indicate that more than one receptor class is involved; otherwise, at a given response level below saturation, response shape should be constant (see Cronin, 1989). Thus, UV photosensitivity is not due either to absorption by a *cis*-peak of the rhodopsin, or to the action of a sensitizing pigment upon a rhodopsin absorbing at longer wavelengths (see Kirschfeld, Franceschini & Minke, 1977).

An unusual feature of UV photoreceptors in mantis shrimps (at least in *G. oerstedii*) is their polarizational sensitivity (PS) in the two most ventral ommatidial rows of the midband (Fig. 2), where R8 cells have unidirectional microvilli (Marshall *et al.*, 1991a). The correlation between PS and the presence of unidirectional microvilli in rhabdomeres of the R8 cell is strong evidence for UV photosensitivity in this receptor class. As is typical of rhabdomeric photoreceptors, sensitivity is maximal when the e-vector of the stimulating light is parallel to the microvillar axes, suggesting that the absorbing dipoles are parallel to the axes of microvilli (review: Rossel, 1989). Elsewhere in the retina, stomatopod R8 cells are typically crustacean, having layered, mutually perpendicular microvilli produced by a single four-lobed cell body (see Waterman, 1981).

Spectral sensitivity and visual pigments of the UV photoreceptors

Spectral sensitivity of the pupillary response peaks near 350 nm in *G. oerstedii* (Fig. 3). The shape of the spectral sensitivity curve (particularly the rapid decline above 350 nm) implies that the sensitivity is based on a visual pigment of λ_{max} near 325 nm which is strongly filtered at short wavelengths, probably by the overlying dioptrics (cornea and crystalline cone). If UV vision is spectrally optimized there is a strong implication that very useful information is available at unexpectedly short wavelengths. The animal may have to tolerate the loss of sensitivity at the peak, if the dioptrics cannot be made more transparent at short wavelengths, to retain the rapid falloff of sensitivity in the mid-300 nm range.

Properties of the UV-absorbing visual pigment revealed by microspectrophotometry in several stomatopod species are entirely consistent with the spectral sensitivity measured physiologically in *G. oerstedii*. The implication is that UV photosensitivity is very conservative in the mantis shrimps, both within the retina and across species. This conservatism is in clear contrast to the diversity of visual pigments operating in the visible region of the spectrum. However, these UV rhodopsins appear to absorb at unusually short wavelengths, with λ_{max} near 325–340 nm. Results of all spectrophotometric examinations of invertebrate visual pigments were reviewed about a decade ago by Stavenga and Schwemer (1984); at that time, no visual pigment with λ_{max} below 345 nm had been found. More recent results do not change this (Muri & Jones, 1983; Schwind, Schlect & Langer, 1984) and the results of numerous electrophysiological measurements of UV photosensitivity are also consistent with mid-300-nm visual pigments (Menzel, 1979; Hardie, 1984; Arikawa, Inokuma & Eguchi, 1987; Peitsch, Fietz, Hertel, de Souza, Ventura & Menzel, 1992). Vertebrate UV photopigments, of course, typically absorb maximally near 360 nm (Hárosi & Hashimoto, 1983; Bowmaker & Kunz, 1987; Hawryshyn & Hárosi, 1991; Jacobs, 1992).

Metarhodopsins ($\lambda_{max} \approx 460$ nm) of mantis shrimp UV rhodopsins are typical UV metapigments, which normally have peak absorption between 460 and 486 nm (Stavenga & Schwemer, 1984; Muri & Jones, 1983; Schwind *et al.*, 1984). MSP results are quite noisy in the region of the rhodopsin peak, making it difficult to determine the R/M extinction ratio. The best-fit difference spectrum in Fig. 4 assumes an extinction ratio of 1.0, which is low for bistable visual pigment systems (Muri & Jones, 1983; Schwind *et al.*, 1984; Cronin & Goldsmith, 1982; Cronin & Forward, 1988). The chromophore of UV visual pigments of stomatopods is almost certainly retinal$_1$, since this is the only retinoid yet found in mantis shrimp compound eyes (Goldsmith & Cronin, 1993).

Significance of UV vision

Vision in the UV region of the spectrum has been assigned a number of functions. Among these are (i) to extend color vision into the UV spectrum for trichromatic, tetrachromatic, or pentachromatic vision (Menzel, 1979; Menzel & Backhaus, 1989; Langer, Hamann & Meinecke, 1979; Arikawa *et al.*, 1987; Burkhardt, 1989; Neumeyer, 1992; Jacobs, 1992); (ii) to detect color-specific features of conspecifics or food plants (Bernard & Remington, 1991; Chittka & Menzel, 1992); (iii) in association with polarization sensitivity, to enable navigation using celestial UV polarization patterns (review: Rossel, 1989); (iv) in association with other spectral receptors, to enhance the analysis of polarized light (Hawryshyn & McFarland, 1987); and (v) to detect specific surfaces, such as water, also in association with polarized-light vision (Schwind, 1984). Mantis shrimps certainly could use UV photosensitivity for imaging, since the retinal hemispheres have extended visual fields and include UV receptors in all ommatidia. But it is not yet clear whether the UV signal gathered by the R8 cells is neurally compared with incoming signals from the main rhabdoms, thus incorporating UV sensitivity into the polychromatic system operating at wavelengths beyond 400 nm. Like many other animals (but apparently unlike other crustaceans), mantis shrimps combine UV sensitivity with polarization sensitivity in a subset of their UV photoreceptors, those of midband Rows 5 and 6. As discussed next, this arrangement could be used to enhance contrast of objects seen in the UV.

Goldsmith (1990) has cautioned us that it is naive and anthropocentric to ask "what do UV photoreceptors *do* for animals?" Nevertheless, expansion of their visual world into the UV may offer mantis shrimps special advantages. The submarine environment is visually low in contrast, mostly because scattering of short-

wavelength light clouds and veils images of objects (Lythgoe, 1979). But at short ranges, the scattered light—of which UV is a major component—can be used to silhouette an object that otherwise would be effectively camouflaged. For example, many fish are silvery to reflect light in such a way that they are invisible against the underwater light field (Denton & Nicol, 1965, 1966). This type of camouflage is worthless against a scattering background. Furthermore, because the reflection is produced by constructive interference, it is inherently less effective in the UV. Therefore, even well-camouflaged fish are easily seen against the bright UV background (Fig. 6). When combined with the analysis of polarization, a visual system like that of the mantis shrimp could potentially break almost any imaginable camouflage system (see also Moody & Parriss, 1960). If this view is correct, UV vision does indeed "do" something for a mantis shrimp. It gives the shrimp a better chance to see animals in the water surrounding and above its burrow, thus providing it the early opportunity to decide whether the best next response is to eat, avoid, or ignore them.

REFERENCES

Arikawa, K., Inokuma, K. & Eguchi, E. (1987). Pentachromatic visual system in a butterfly. *Naturwissenschaften, 74,* 297–298.

Bernard, G. D. (1987). Spectral characterization of butterfly L-receptors using extended Dartnall/MacNichol template functions. *Journal of the Optical Society of America A, 4,* P123.

Bernard, G. D. & Remington, C. L. (1991). Color vision in *Lycaena* butterflies: Spectral tuning of receptor arrays in relation to behavioral ecology. *Proceedings of the National Academy of Sciences, U.S.A., 88,* 2783–2787.

Bernard, G. D. & Stavenga, D. G. (1979). Spectral sensitivities of retinular cells measured in intact, living flies by an optical method. *Journal of Comparative Physiology, 134,* 95–107.

Bernard, G. D. & Wehner, R. (1980). Intracellular optical physiology of the bee's eye—I. Spectral sensitivity. *Journal of Comparative Physiology, 137,* 193–203.

Bowmaker, J. K. & Kunz, Y. W. (1987). Ultraviolet receptors, tetrachromatic colour vision and retinal mosaics in the brown trout (*Salmo trutta*): Age-dependent changes. *Vision Research, 27,* 2102–2108.

Burkhardt, D. (1989). UV vision: A bird's eye view of feathers. *Journal of Comparative Physiology A, 164,* 787–796.

Cavenaugh, G. M. (1956). *Formulae and methods of the marine biological laboratory chemical room.* Woods Hole, Mass.

Chapman, R. M. & Lall, A. B. (1967). Electroretinogram characteristics and the spectral mechanisms of the median ocellus and the lateral eye in *Limulus polyphemus. Journal of General Physiology, 50,* 2267–2287.

Chittka, L. & Menzel, R. (1992). The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *Journal of Comparative Physiology A, 171,* 171–181.

Cronin, T. W. (1989). Application of intracellular optical techniques to the study of stomatopod crustacean vision. *Journal of Comparative Physiology A, 164,* 737–749.

Cronin, T. W. & Forward, R. B. Jr (1988). The visual pigments of crabs—I. Spectral properties. *Journal of Comparative Physiology A, 162,* 463–478.

Cronin, T. W. & Goldsmith, T. H. (1982). Quantum efficiency and photosensitivity of the rhodopsin-metarhodopsin conversion in crayfish photoreceptors. *Photochemistry and Photobiology, 36,* 447–454.

Cronin, T. W. & King, C. A. (1989). Spectral sensitivity of vision in the mantis shrimp, *Gonodactylus oerstedii,* determined using noninvasive optical techniques. *Biological Bulletin, 176,* 308–316.

Cronin, T. W. & Marshall, N. J. (1989a). A retina with at least ten spectral types of photoreceptors in a stomatopod crustacean. *Nature, 339,* 137–140.

Cronin, T. W. & Marshall, N. J. (1989b). Multiple spectral classes of photoreceptors in the retinas of gonodactyloid stomatopod crustaceans. *Journal of Comparative Physiology A, 166,* 267–275.

Cronin, T. W., Marshall, N. J. & Caldwell, R. L. (1993). Photoreceptor spectral diversity in the retinas of squilloid and lysiosquilloid stomatopod crustaceans. *Journal of Comparative Physiology A, 172,* 339–350.

Cummins, D. R. & Goldsmith, T. H. (1981). Cellular identification of the violet receptor in the crayfish eye. *Journal of Comparative Physiology, 142,* 199–202.

Cummins, D. R., Chen, D.-M. & Goldsmith, T. H. (1984). Spectral sensitivity of the spiny lobster, *Panulirus argus. Biological Bulletin, 166,* 269–276.

Demoll, R. (1909). Über die Augen und der Augenstiele-reflexe von *Squilla mantis. Zoologische Jahrbucher, Abteilung für Anatomie und Ontogenie der Tiere, 27,* 171–212.

Denton, E. J. & Nicol, J. A. C. (1965). Studies on reflexions of light from silvery surfaces of fishes, with special reference to the bleak, *Alburnus alburnus. Journal of the Marine Biological Association of the U.K., 45,* 683–703.

Denton, E. J. & Nicol, J. A. C. (1966). A survey of reflectivity in silvery teleosts. *Journal of the Marine Biological Association of the U.K., 46,* 685–722.

Exner, S. (1891). *Die Physiologie der facettirten Augen von Krebsen und Insecten.* Leipzig: Deuticke.

Franceschini, N. (1975). Sampling of the visual environment by the compound eye of the fly: Fundamentals and applications. In Snyder, A. W. & Menzel, R. (Eds), *Photoreceptor optics* (pp. 98–125). Berlin: Springer.

Frank, T. M. & Case, J. F. (1988). Visual spectral sensitivities of bioluminescent deep-sea crustaceans. *Biological Bulletin, 175,* 261–273.

Goldsmith, T. H. (1990). Optimization, constraint and history in the evolution of eyes. *Quarterly Review of Biology, 65,* 281–322.

Goldsmith, T. H. & Cronin, T. W. (1993). The retinoids of seven species of mantis shrimp. *Visual Neuroscience, 10,* 915–920.

Goldsmith, T. H. & Fernandez, H. R. (1968). Comparative studies of crustacean spectral sensitivity. *Zeitschrift für Vergleichhende Physiologie, 60,* 156–175.

Jacobs, G. H. (1992). Ultraviolet vision in vertebrates. *American Zoologist, 32,* 544–554.

Hardie, R. C. (1984). Properties of photoreceptors R7 and R8 in dorsal marginal ommatidia in the compound eyes of *Musca* and *Calliphora. Journal of Comparative Physiology A, 154,* 157–165.

Hárosi, F. I. & Hashimoto, Y. (1983). Ultraviolet visual pigment in a vertebrate: A tetrachromatic cone system in the dace. *Science, 222,* 1021–1023.

Hawryshyn, C. W. & Hárosi, F. I. (1991). Ultraviolet photoreception in carp: Microspectrophotometrically and behaviorally determined action spectra. *Vision Research, 31,* 567–576.

Hawryshyn, C. W. & McFarland, W. N. (1987). Cone photoreceptor mechanisms and the detection of polarized light in fish. *Journal of Comparative Physiology A, 160,* 459–465.

Horridge, G. A. (1978). The separation of visual axes in apposition compound eyes. *Philosophical Transactions of the Royal Society of London B, 285,* 1–59.

Kirschfeld, K., Franceschini, N. & Minke, B. (1977). Evidence for a sensitizing pigment in fly photoreceptors. *Nature, 269,* 386–390.

Lall, A. B. & Cronin, T. W. (1987). Spectral sensitivity of the compound eyes in the purple land crab, *Gecarcinus lateralis. Biological Bulletin, 173,* 398–406.

Langer, H., Hamann, B. & Meinecke, C. C. (1979). Tetrachromatic visual system in the moth *Spodoptera exempta. Journal of Comparative Physiology, 129,* 235–239.

Lythgoe, J. N. (1979). *The ecology of vision.* Oxford: Clarendon Press.

Marshall, N. J. (1988). A unique colour and polarization vision system in mantis shrimps. *Nature, 333*, 557–560.

Marshall, N. J., Land, M. F., King, C. A. & Cronin, T. W. (1991a). The compound eyes of mantis shrimps (Crustacea, Hoplocarida, Stomatopoda)—I. Compound eye structure: The detection of polarised light. *Philosophical Transactions of the Royal Society of London B, 334*, 33–56.

Marshall, N. J., Land, M. F., King, C. A. & Cronin, T. W. (1991b). The compound eyes of mantis shrimps (Crustacea, Hoplocarida, Stomatopoda)—II. Colour pigments in the eyes of Stomatopod crustaceans: Polychromatic vision by serial and lateral filtering. *Philosophical Transactions of the Royal Society of London B, 334*, 57–84.

Martin, F. G. & Mote, M. I. (1982). Color receptors in marine crustaceans: A second spectral class of retinular cell in the compound eyes of *Callinectes* and *Carcinus*. *Journal of Comparative Physiology, 145*, 549–554.

Menzel, R. (1979). Spectral sensitivity and color vision in invertebrates. In Autrum, H. (Ed.), *Handbook of sensory physiology, Vol. VII, Part 6A. Vision in invertebrates. A: Invertebrate photoreceptors* (pp. 501–580). Berlin: Springer.

Menzel, R. & Backhaus, W. (1989). Color vision in honey bees: Phenomena and physiological mechanisms. In Stavenga, D. G. & Hardie, R. C. (Eds), *Facets of vision* (pp. 281–297). Berlin: Springer.

Moody, M. F. & Parriss, J. R. (1960). The discrimination of polarized light by *Octopus*. *Nature, 186*, 839–840.

Muri, R. B. & Jones, G. J. (1983). Microspectrophotometry of single rhabdoms in the retina of the honeybee drone (*Apis mellifera*). *Journal of General Physiology, 82*, 469–496.

Neumeyer, C. (1992). Tetrachromatic color vision in goldfish: Evidence from color mixture experiments. *Journal of Comparative Physiology A, 171*, 639–649.

Nolte, J. & Brown, J. E. (1970). The spectral sensitivities of single receptor cells in the lateral, median and ventral eyes of normal and white-eyed *Limulus*. *Journal of General Physiology, 55*, 787–801.

Peitsch, P., Fietz, A., Hertel, H., de Souza, J., Ventura, D. F. & Menzel, R. (1992). The spectral input systems of hymenopteran insects and their receptor-based colour vision. *Journal of Comparative Physiology A, 170*, 23–40.

Rossel, S. (1989). Polarization sensitivity in compound eyes. In Stavenga, D. G. & Hardie, R. C. (Eds), *Facets of vision* (pp. 298–316). Berlin: Springer.

Schiff, H. (1963). Dim light vision of *Squilla mantis* L. *American Journal of Physiology, 205*, 927–940.

Schwind, R. (1984). Evidence for true polarization vision based on a two-channel analyzer system in the eye of the water bug, *Notonecta glauca*. *Journal of Comparative Physiology A, 154*, 53–57.

Schwind, R., Schlect, P. & Langer, H. (1984). Microspectrophotometric characterization and localization of three visual pigments in the compound eye of *Notonecta glauca* L. (Heteroptera). *Journal of Comparative Physiology A, 154*, 341–346.

Smith, R. C. & Baker, K. S. (1979). Penetration of UV-B and biologically effective dose-rates in natural waters. *Photochemistry and Photobiology, 29*, 311–323.

Smith, K. C. & Macagno, E. R. (1990). UV photoreceptors in the compound eye of *Daphnia magna* (Crustacea, Branchiopoda). A fourth spectral class in single ommatidia. *Journal of Comparative Physiology A, 166*, 597–606.

Stavenga, D. G. (1979). Pseudopupils of compound eyes. In Autrum, H. (Ed.), *Handbook of sensory physiology, Vol. VII, Part 6A. Vision in invertebrates. A: Invertebrate photoreceptors* (pp. 357–439). Berlin: Springer.

Stavenga, D. G. & Schwemer, J. (1984). Visual pigments of invertebrates. In Ali, M. A. (Ed.), *Photoreception and vision in invertebrates* (pp. 11–61). New York: Plenum Press.

Wald, G. & Krainin, J. M. (1963). The median eye of *Limulus*: An ultraviolet receptor. *Proceedings of the National Academy of Sciences, U.S.A., 50*, 1011–1017.

Waterman, T. H. (1981). Polarization sensivitity. In Autrum, H. (Ed.), *Handbook of sensory physiology, Vol. VII, Part 6B. Vision in invertebrates. B: Invertebrate visual centers and behavior I* (pp. 281–469). Berlin: Springer.

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