Spectral Sensitivities of Wolf Spider Eyes

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ABSTRACT   ERG's to spectral lights were recorded from all eyes of intact wolf spiders. Secondary eyes have maximum relative sensitivities at 505–510 nm which are unchanged by chromatic adaptations. Principal eyes have ultraviolet sensitivities which are 10 to 100 times greater at 380 nm than at 505 nm. However, two animals' eyes initially had greater blue-green sensitivities, then in 7 to 10 wk dropped 4 to 6 log units in absolute sensitivity in the visible, less in the ultraviolet. Chromatic adaptations of both types of principal eyes hardly changed relative spectral sensitivities. Small decreases in relative sensitivity in the visible with orange adaptations were possibly retinomotor in origin. Second peaks in ERG waveforms were elicited from ultraviolet-adapted principal eyes by wavelengths 400 nm and longer, and from blue-, yellow-, and orange-adapted secondary eyes by wavelengths 580 nm and longer. The second peaks in waveforms were most likely responses of unilluminated eyes to scattered light. It is concluded that both principal and secondary eyes contain cells with a visual pigment absorbing maximally at 505–510 nm. The variable absolute and ultraviolet sensitivities of principal eyes may be due to a second pigment in the same cells or to an ultraviolet-absorbing accessory pigment which excites the 505 nm absorbing visual pigment by radiationless energy transfer.

It is now well-known that many insects can see color, but it remains to be proven that another group of arthropods, the spiders, also see color. Peckham and Peckham (1887) thought spiders could see color, since wolf spiders came to rest preferentially under red glass as opposed to blue, yellow, or green glasses. Frings (1941) was unable to repeat the Peckhams' results; he used equally illuminated as well as equally transmitting red, yellow, or blue cellophanes. Schlegtendal (1934) used moving colored stripes to elicit optomotor responses in a variety of arthropods. She was unable to elicit a consistent behavioral response from wolf spiders, much less a color preference, and concluded only that the moving stripes released an escape reflex.

The most thorough work on spider color responses is that of Kästner (1950). He used a behavioral approach based on the observation that jumping spiders (*Evarcha falcata*) would preferentially leap upon and ascend a
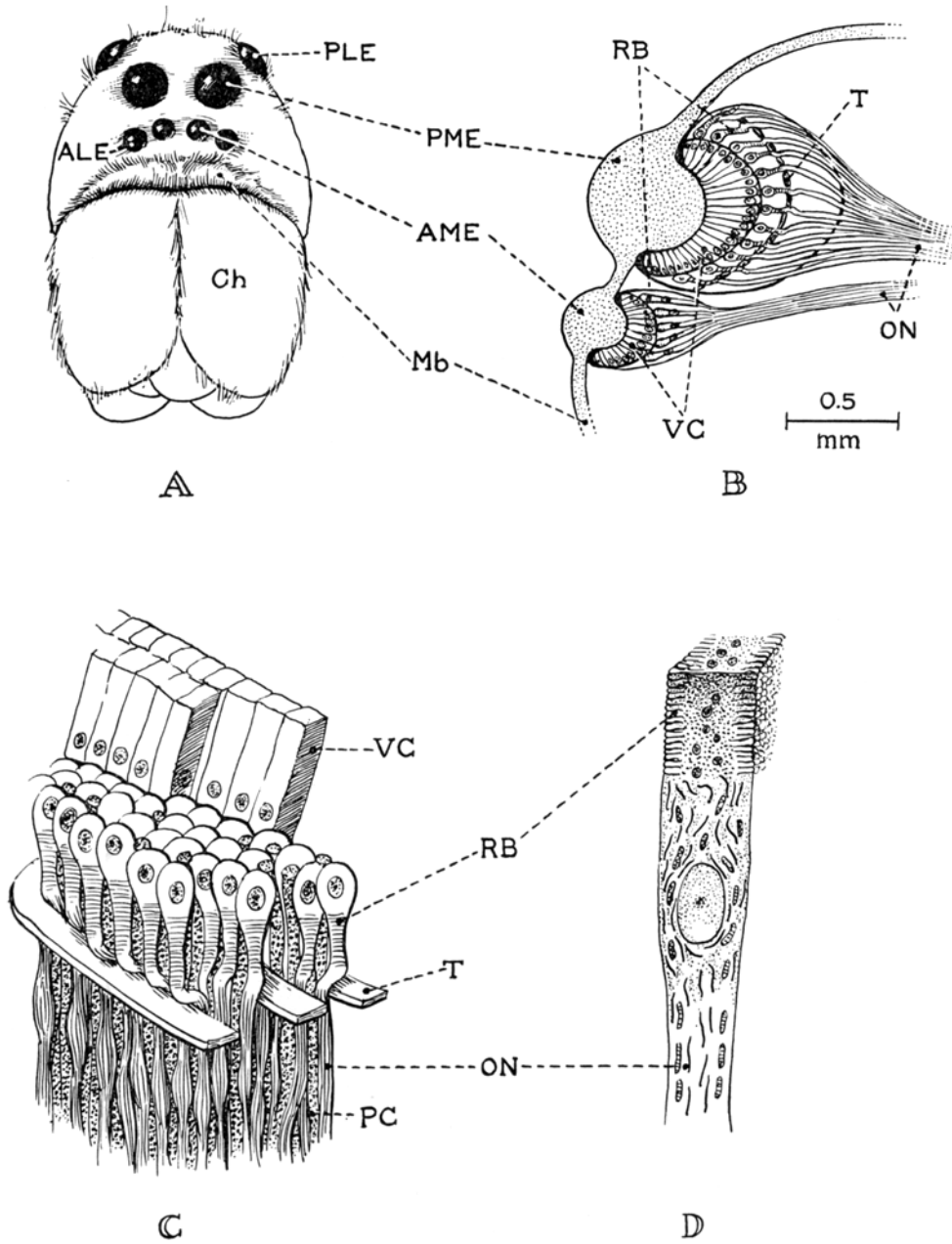
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FIGURE 1.   Anatomy of wolf spider eyes. A. drawing of the external anatomy of the head of *Lycosa miami*, seen from the front. *AME*, anterior median eye, *ALE*, anterior lateral eye, *PME*, posterior median eye, *PLE*, posterior lateral eye, *Mb*, articulation membrane separating carapace, upon which the eyes are located, from the chelicerae, *Ch*. B. diagrammatic, composite drawing to scale of parasagittal sections of *Lycosa miami*. Only a few retinal cells are shown in each eye. *VC*, vitreous cells, *RB*, rhabdomes, *T*,

stationary striped wall as opposed to a featureless wall. The reaction was very strong (85–92 %) when white stripes alternated with black, and became weaker as gray stripes were substituted for white. On the other hand, when blue stripes or orange stripes were presented alternately with any of 26 different shades of gray stripes, the animals always reacted as strongly as they had to the black and white stripes. Kästner argued that the blue or orange stripes did not appear gray to the spiders, else they would have been confused somewhat with at least one of the gray shades. Therefore, he concluded, the spiders saw the color and not just the brightness of the blue and orange stripes. That is, they had color vision. He also concluded that his spiders used the fine resolution of their anterior median (principal) eyes for the stripe, and hence, color discriminations. (A description of the various spider eyes will be given below and in Fig. 1.)

These conclusions of Kästner were the original impetus for ERG studies of spider spectral sensitivities in this laboratory. It was reasoned that if anterior median eyes of jumping spiders could distinguish colors, they must contain at least two spectrally different visual cell populations, one or the other of which would be selectively adaptable by chromatic lights. Preliminary experiments on anterior median eyes of jumping spiders showed, however, that chromatic (blue or orange) light adaptations which reduced sensitivities 1 log unit or so did not change relative spectral sensitivities at all (DeVoe and Zvargulis, 1967; see below). This would not have been expected had one cell population been selectively more adapted than the other. On the other hand, there was observed in both light- and dark-adapted eyes a higher sensitivity to ultraviolet wavelengths (less than 420 nm) than to visible wavelengths, where there was a sensitivity maximum at 530–535 nm. With the equipment then available, the maximum ultraviolet sensitivity could not be measured, however.

These experiments from this laboratory suggested that differences in ultraviolet reflectivities of stripes might have aided Kästner's (1950) jumping spiders in their claimed color discriminations. It therefore seemed important to study the ultraviolet sensitivities of anterior median eyes further, as well

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tapetum (not found in the *AME*), *ON*, optic nerves. The optic nerve fibers leave the *PME* via fenestrations in the eye cup, shown as a heavy line. The vitreous cells and hypodermal cells (not shown) are pigmented at the margins of the lenses, giving the effect of an iris. C. diagrammatic, perspective drawing of the retina of a secondary eye, based on Baccetti and Bedini (1964). Rhabdomes, *RB*, are drawn lined solely to indicate their positions; otherwise, their structure is microvillar as in D. *PC*, pigment cells, *T*, tapetum, *ON*, optic nerve, *VC*, vitreous cells. D. diagrammatic, perspective drawing of a visual cell from the periphery of an *AME*, based on the work of Melamed and Trujillo-Cenoz (1966). Central cells are pentagonal. Sizes of retinal cells of all eyes in spiders used for the present study were on the order of 12 μM across at the rhabdomes.

as to find out whether the potential for color vision might exist in the six other (secondary) eyes. As our jumping spiders were quite small (about 1 cm body length) and therefore difficult to record from electrically, these further experiments have been done on wolf spiders, which are also vagabonds but are larger. Some preliminary results of spectral sensitivity measurements on posterior median (secondary) eyes of wolf spiders have previously been published (DeVoe and Zvargulis, 1967).

The Nomenclature and Structure of Spider Eyes   In general, spiders have two rows of four ocelli each. As illustrated in Fig. 1 A from one of the wolf spiders used in this work, these eyes are called anterior median (*AME*), anterior lateral (*ALE*), posterior median (*PME*), and posterior lateral (*PLE*). In parasagittal section, the eyes appear as in the diagrammatic, composite drawing of Fig. 1 B. The lenses are cuticular thickenings, directly behind which lie the columnar vitreous cells (*VC*). The anterior median eyes, called principal eyes, have one retinal structure, and all others have another (Scheuring, 1914; Baccetti and Bedini, 1964; Melamed and Trujillo-Cenoz, 1966). The differences are of embryological origin (Lambert, 1909; Homann, 1955) and exist primarily in the positions of the rhabdomes. In the anterior median eyes, the rhabdomes (*RB*) lie directly behind the vitreous cells, followed by the cell body and then the optic nerve fibers (*ON*). Fig. 1 D, based on the work of Melamed and Trujillo-Cenoz (1966), is a schematic drawing of a visual cell from the periphery of a wolf spider anterior median eye. The rhabdomes (*RB*) here, as in the other eyes, are composed of microvilli.

In all the other eyes, called secondary eyes, the cell bodies lie directly behind the vitreous cells, followed by the rhabdomes and then the optic nerve fibers. This is illustrated in Fig. 1 C, based on the work of Baccetti and Bedini (1964). There exists an elaborate, sinuous tapetum (*T*), with the visual cells placed so that the rhabdomes lie directly distal to the tapetal strips while the cell axons bend around to pass between these strips. In this way, light which passes down the rhabdomes is reflected back by the tapetum, while light which does not enter the rhabdomes is presumably absorbed by shielding pigments (*PC*) between the rhabdomes. (The rhabdomes of anterior median eyes do not appear to be separated by shielding pigments. However, see further discussion of this point later in this paper.) From both eyes, the optic nerves proceed without synapse to the brain, or supraesophageal ganglion, which in our wolf spiders was about 3 mm away.

METHODS

Preparation of the Animal   As described previously (DeVoe, 1967 *a*), intact animals were nondestructively waxed into a clamp from which they could be released after each experiment. This allowed us to use one animal in five experiments, and

two animals in two experiments each. Specimens of *Lycosa baltimoriana*, *L. miami*, and *L. carolinensis* were used. In some experiments, black paint covered all but the illuminated eye(s), but the results were not different.

Electrical contract was made with the illuminated eye by a glass saline-filled pipette of 0.3–0.4 mm tip diameter. A similar but electrically indifferent pipette touched a small wound far back on the carapace. Silver-silver chloride electrodes, not exposed to the light, made contact with the pipettes via salt bridges. All recording used a DC-coupled, differential, 20 × preamplifier and a DC-coupled Tektronix 502 oscilloscope (Tektronix, Inc., Beaverton, Oreg.). The two oscilloscope traces of response and shutter monitor were photographed with a Grass C-4 oscillograph camera (Grass Instrument Co., Quincy, Mass.). Amplitudes and waveforms of responses were measured off the projected films. Temperature during the experiments was maintained at 20–22°C.

Optical Stimulation   The triple-beam optical stimulator used is schematized in its final form in Fig. 2. Details of the stimulator are given in the caption, but several points need to be mentioned. Wandering of the xenon arc (Xe) was monitored by reflecting a small part of its output onto a pair of silicon solar cells (SS), whose junction was conjugate with the entrance slit (SL_1) of the monochromator. The xenon lamp was initially focused and centered mechanically upon the entrance slit. Then, the cover glass beam splitter (BS_1) and balance potentiometer (B) were adjusted to give a center zero reading on the meter (M). Subsequent shifts in xenon arc position could then be corrected by mechanically moving the lamp horizontally until the meter again read zero. Such corrections were often necessary during the course of an experiment.

It was also desired to monitor the movements of the test-flash shutter (SH_1) with a photodetector independent of monochromator wavelength and yet without the danger of stimulating the eye with stray light. The solution was to use a gallium arsenide diode light source ($GaAs$: Texas Instruments T1XL01, Texas Instruments, Inc., Dallas, Tex.), which emits maximally at 900 nm. The diode shone at an angle through the shutter center onto a spectrally matching silicon phototransistor (Texas Instruments LS-400).

Finally, all combined beams were focused onto a fiber optic (FO), whose other end was placed upon the illuminated eye. For experiments on the relatively large posterior median eyes, this fiber optic was a 31 fiber bundle of 0.5 mm diameter (LGM or ULGM: American Optical Company, Southbridge, Mass.). Stimulation of all other eyes was accomplished with a single ULGM fiber of 75 μm diameter sealed with epoxy into a length of 31 gauge hypodermic tubing. The glass achromatic condenser was achromatic only to about 370–380 nm, resulting in severe attenuation of intensities at 350 and 360 nm. The low intensities at these latter wavelengths made their calibrations less certain than at longer wavelengths.

Calibrations   Most radiometric calibrations were made using a Kettering radiometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). For the experiments on the posterior median eyes, the radiometer receiver was placed directly at the end of the fiber optic bundle, most of whose light was intercepted. For the ex-

periments with the single fiber optic, there was insufficient intensity at its end for direct calibration and the following, more involved calibration was used. First a photomultiplier tube (Dumont No. 6291, S-11 response) was placed at the end of the fiber optic and the calibrated wedges were adjusted at each wavelength to give
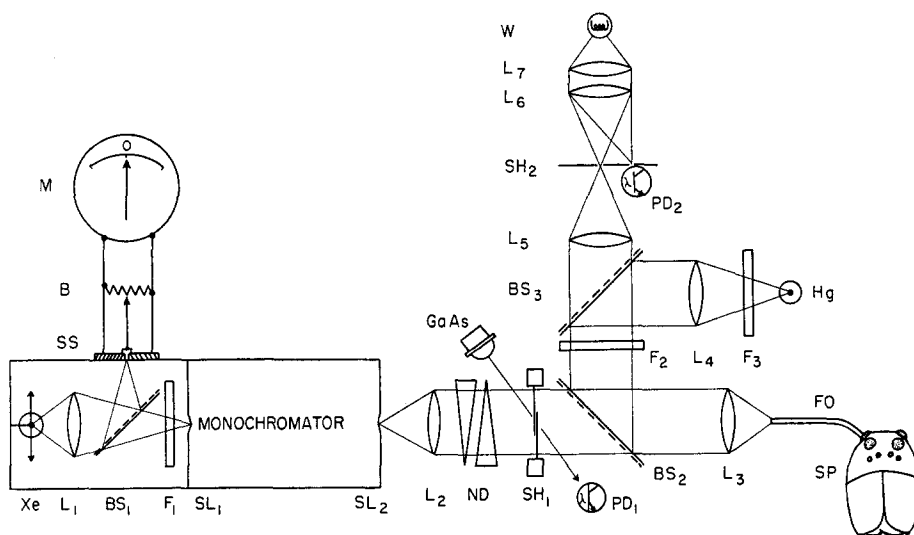

FIGURE 2. Schematic diagram of the optical stimulator. Xe, 150 w xenon arc lamp (a tungsten lamp was used in early experiments); L_1, quartz lens; BS_1, cover glass beam splitter; F_1, glass heat and color filters to reduce stray light (see Table I); SL_1, entrance slit of monochromator; SS, silicon solar cells conjugate with SL_1 (see text); B, balance potentiometer; M, zero-center microammeter; monochromator, Bausch and Lomb high intensity monochromator with visible grating of 6.5 nm/mm dispersion; SL_2, 1.5 mm exit slit with 9.6 nm bandwidth (in early experiments a 3 mm exit slit with 19.2 nm bandwidth was used); L_2, Bausch and Lomb quartz-fluorite achromatic condenser lens; ND, circular, M type, 0–8 neutral density wedges (Eastman Kodak Company); SH_1, Wollensak heavy-duty, self-cocking shutter driven by solenoid; $GaAs$, gallium arsenide light diode (see text); PD_1, phototransistor; BS_2, nonabsorbing beam splitter (Edmund Scientific Company, Barrington, N.J.); L_3, Bausch and Lomb achromatic substage condenser with front element removed; FO, fiber optic (see text); SP, spider. W, 45 w quartz-iodide lamp; L_6, L_7, and L_5, collimating lenses; SH_2, electromagnetic shutter; PD_2 : phototransistor; BS_3, nonabsorbing beam splitter; Hg, Gates MLA-85DC mercury arc lamp (G. W. Gates and Co., Franklin Square, N.Y.); F_3, Wratten 18A filter to isolate the mercury 365 nm lines; L_4, quartz collimating lens.

the same photocurrent. Then, the fiber optic was removed and the face of the photomultiplier tube was placed where the entrance of the fiber optic had been. The wedge setting needed to give the same photocurrent at each wavelength was again found. The differences in wedge settings thus gave the attenuation at each wavelength due to the single fiber optic. Finally, the radiometer receiver was placed where the entrance of the fiber optic had been and the full radiometric output of the monochromator was measured. The image of the monochromator exit slit, which was

focused upon the radiometer receiver, was sufficiently small so that most or all of it fell on the receiver. The receiver was by no means evenly illuminated, however. Thus two geometric errors, uneven receiver illumination and possibly incomplete interception of all light, allow us to give only the minimum quantum flux upon the eye. Quantum fluxes are given in terms of quanta/sec at the eye (that is, issuing from the end of the fiber optic), the radiometer receiver area having been taken into account.

Additional calibrations were made using an Epply thermopile (Epply Laboratories, Newport, R. I.) placed at the end of the single fiber optic. The results were essentially the same as with the radiometer, except for a nearly constant, multiplicative scale factor presumably due to uneven illumination of the thermopile. There was insufficient intensity to use the thermopile below 400 nm, however.

TABLE I

Wavelength range	Colored filter	Heat filter			
nm					
350, 360	Schott* UG-1	Schott KG-1		Schott WG-5	
370–430	Corning‡ 7-59	"	"	"	"
440–530	Corning 4-76	Fish-Shurman§		XUR-96	
540–650	Corning 3-69	"	"	"	"
660–720	Corning 2-63	"	"	"	"

Colored glass filters used to reduce stray light from the monochromator
* Jenaer Glaswerk Schott und Gen., Mainz, West Germany, distributed by Fish-Shurman Corp., New Rochelle, N.Y.
‡ Corning Glass Works, Corning, N.Y.
§ Fish-Shurman, New Rochelle, N.Y.

Monochromator settings were checked using a Gates mercury arc lamp (George Gates and Company, Franklin Square, N. Y.) and were found to be accurate to ±1 nm.

Stray Light Broad-band glass color filters (F_1 in Fig. 2) were used to restrict stray light, and heat filters were used to protect the glass filters. Table I gives the filters and the wavelength bands in which they were used. Early experiments without these color filters showed stray light to be a problem only below 380 nm.

Analysis Methods In all experments upon the secondary eyes and in some upon principal eyes, responses were recorded at each wavelength at a number of intensities. The intensities needed to obtain a constant response were then interpolated or extrapolated from a more extensive stimulus-response curve (for justification, see Results). Later experiments on the principal eyes used a method reported by Goldsmith and Ruck (1958). One of us observed the oscilloscope screen and controlled timing of stimuli while the other adjusted stimulus intensities at each wavelength until the observer signalled that a criterion response (50 or 100 μv) had been met. In some experiments these responses were also photographed, and stimulus intensities were corrected for small deviations from the criterion response. In all experiments, intensities were presented to the eye in random order, and control wavelengths

(usually 500–520 nm or 370–380 nm) were repeated at intervals. All spectral sensitivities plotted have been corrected for drifts in controls.

RESULTS

Secondary Eyes (Nebenaugen)

STIMULUS-RESPONSE CURVES   Relations between amplitudes of responses from a posterior median eye and flash intensities at a number of different
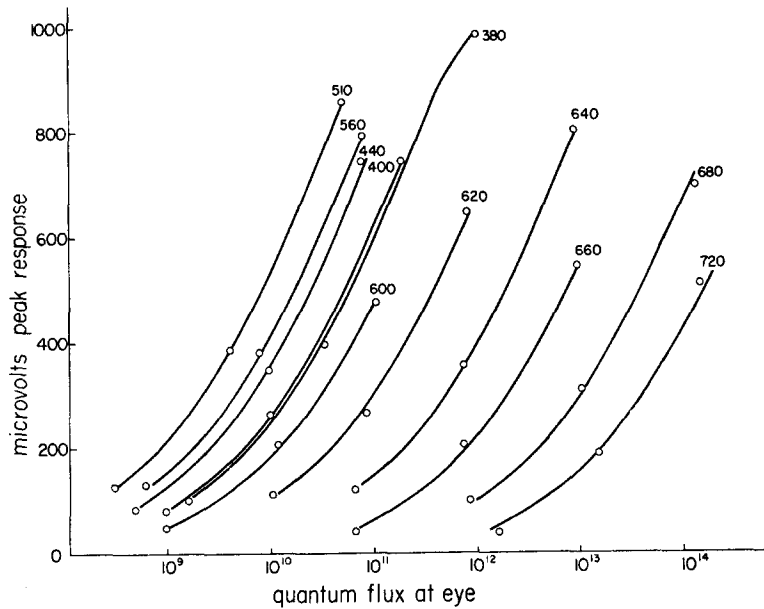

FIGURE 3.   Stimulus-response curves for a dark-adapted posterior median (secondary) eye. Abscissa, number of quanta/sec emergent from the end of the fiber optic touching the eye. Ordinate, peak microvolt responses to 15 msec flashes of the wavelengths, in nanometers, indicated on each curve. The open circles are experimental points. The solid lines are one curve which has been shifted along the intensity axis (abscissa) to give the best fit by eye to each set of points. For clarity, experimental points for a number of wavelengths have been omitted. Quartz-iodide light source in monochromator, half bandwidth of 19.2 nm, no colored filters to reduce stray light. *Lycosa baltimoriana.*

wavelengths are shown in Fig. 3 as open circles. Such stimulus-response relations have been used for two purposes, first, for determining spectral sensitivities, as in Fig. 4, and second, for seeing whether there are any wavelength-dependent variations. Systematic variations of stimulus-response curve shapes with wavelength in compound eyes of whirligig beetles *Dineutes* (Bennett, 1967) and in median ocelli of *Limulus* (Chapman and Lall, 1967) have been taken as evidence for color mechanisms in these eyes, but so far, they appear to be exceptional. When single cell studies of insect eyes have re-

vealed cells with different spectral responses, the stimulus-response curves of all cells have been the same within experimental error (Burkhardt, 1962; Autrum and von Zwehl, 1964; Bennett, Tunstall, and Horridge, 1967; Bruckmoser, 1968). Systematic variations in stimulus-response curves with wavelength have always been looked for in responses from spider eyes, and in all eyes, they have been consistently absent. Thus in Fig. 3 for a dark-adapted posterior median eye, all data points (open circles) fall on a single curve (the solid line) which has been slid horizontally along the abscissa (quantum flux at the eye) to give the best fit by eye to the points at each wavelength. Other experiments gave similar results.

SPECTRAL SENSITIVITIES   In Fig. 3, the distances along the abscissa which the curve had to be moved from wavelength to wavelength give the spectral sensitivity of the eye. Spectral sensitivities of a different animal are shown in Fig. 4. The ordinate gives the quantum fluxes at each wavelength needed to elicit 130 μv responses. As Fig. 3 shows, however, the shapes of the spectral sensitivity curves are clearly independent of the criterion response amplitude chosen.

Curves in Fig. 4, from both dark- and light-adapted eyes, have maximum sensitivities near 510 nm and match a Dartnall 510 nomogram curve (heavy lines) (Dartnall, 1953) for wavelengths longer than about 460 nm. In some experiments, the fit was good down to about 400 nm.

Chromatic light adaptation does not selectively adapt the eye, however. The thin lines in Fig. 4 were drawn freehand to the dark-adapted data (upper points), then simply moved down by 2.4 log units, the amount by which all the chromatic adaptations reduced the sensitivity at 500–510 nm. This same curve fits both dark- and light-adapted results; there are no changes in relative spectral sensitivities due to chromatic adaptations. This confirms results obtained earlier (DeVoe and Zvargulis, 1967), where only 1 log unit of chromatic adaptation was used. In other ERG studies of arthropod eyes, chromatic adaptations of 1–2 log units have often been quite selective, revealing the existence of at least two color mechanisms (Walther, 1958; Walther and Dodt, 1959; Goldsmith, 1960; Wald and Krainen, 1963; Chapman and Lall, 1967; Bennett, 1967; Wald, 1968). However, we have chromatically light-adapted one posterior median eye by as much as 4.7 log units, and we still found no changes in relative spectral sensitivities compared to the same eye dark-adapted. From this we conclude that there is only one type of spectrally responding cell in wolf spider posterior median eyes (and presumably in the other secondary eyes as well; see below). Most likely, this cell type contains only a single visual pigment absorbing maximally near 510 nm, to judge from the nomogram curves in Fig. 4. Posterior median eyes alone, then, have no potential ability to distinguish colors.

DOUBLE-PEAKED WAVEFORMS OF RESPONSES   Waveforms of all responses from dark-adapted posterior median eyes more or less match each other, and we have found no consistent effects of wavelength on waveforms of these re-
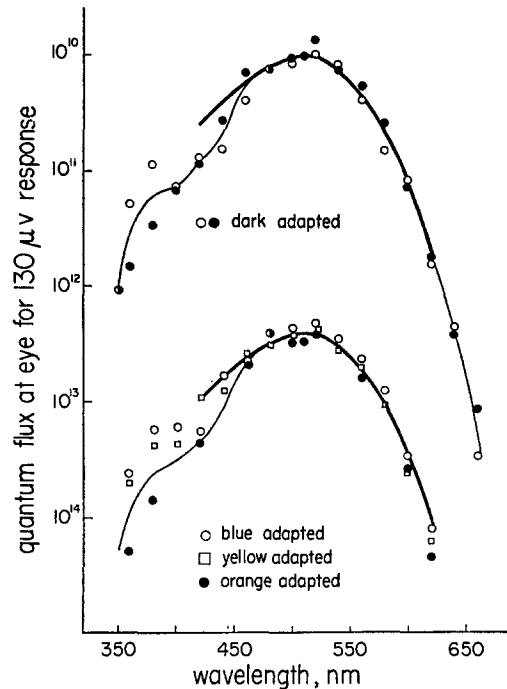

FIGURE 4.   Spectral sensitivities of dark-and light-adapted posterior median eyes. The abscissa gives the wavelengths of flashes in nanometers. The ordinate gives the intensities, as quanta/sec incident upon the eye, required to elicit 130 μv peak responses to 15 msec flashes. All results are taken from two experiments upon the two posterior median eyes of one animal. Closed circles, left posterior median eye; open circles and squares, right posterior median eye. The left eye was 0.2 log unit more sensitive than the right eye. The ordinate refers to flux values for the right eye, and points for the left eye have all been shifted down by 0.2 log unit. The heavy lines are a 510 nm nomogram curve. The thin lines were fitted by eye to the remaining dark-adapted points, then shifted down 2.4 log units. The following filters were used in the tungsten beam (at F_2, Fig. 2): blue-adapted, Schott BG-25 (passes 350–460 nm); yellow-adapted, Schott GG-495 (passes all wavelengths 495 nm and longer); orange-adapted, Schott OG-550 (passes all wavelengths 550 nm and longer). Neutral density filters and tungsten lamp current adjustments were used to reduce the sensitivity by 2.4 log units at 500 nm equally for all adapting colors. Lycosa baltimoriana.

sponses. Some examples of waveforms of responses from dark-adapted eyes are illustrated in the left column of Fig. 5. These responses are simple, cornea-negative potentials, the same as are seen with white-light stimulation (DeVoe, 1967 a).

When we blue- (or yellow- or orange-) adapted these eyes, however, we noticed that long wavelengths of light now elicited more complex responses than did shorter wavelengths. This is illustrated in the middle column of Fig. 5. We will call the more complex responses double-peaked responses, and will distinguish in what follows between the first peaks, seen in responses to all
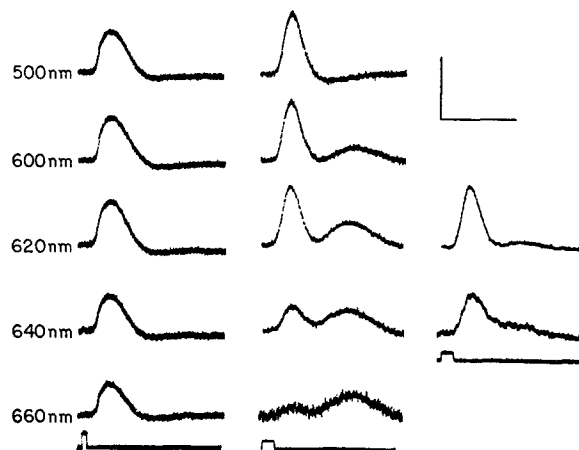

FIGURE 5. Double-peaked responses to long wavelengths in a light-adapted, posterior median eye. Left column, responses of the dark-adapted eye to wavelengths indicated to the left. All responses have the same waveforms and amplitudes. For this column, the vertical calibration mark at the upper right represents 200 μv, the horizontal mark 250 msec. Middle column, responses of the blue-adapted eye to the same wavelengths. Second peaks present at wavelengths of 580 nm (not shown) and longer are absent in the response to 500 nm, where there is an undershoot instead. Vertical calibration, 200 μv except for the bottom trace, where it is 100 μv. Horizontal calibration is 100 msec for all records. Right column, responses of the same eye equally adapted by a 615 nm background light, obtained with an Optics Technology Monopass interference filter (Optics Technology, Inc., Palo Alto, Calif.). The second peaks are largely suppressed. Vertical and horizontal calibrations, 200 μv and 100 msec, respectively. The phototransistor records of 15 msec shutter openings at the bottom of each column apply to all records in that column. Upward deflection of all responses corresponds to corneal negativity with respect to an indifferent electrode. *Lycosa baltimoriana.*

wavelengths, and the second peaks seen (in the posterior median eyes) in responses to wavelengths 580 nm and longer. It is always possible to match the first peak of every long wavelength response with the waveform of an equal-amplitude response to a wavelength in the range 440–560 nm. The first peaks in the long wavelength responses thus appear to originate in the same cells giving the responses to wavelengths 560 nm or shorter. A reasonable assumption, then, is that the second peaks are simply additional potentials adding on to the first peaks and that they are generated by a second group of cells.

Inspection of Fig. 5 reveals that the second peaks have much longer latencies

than the first peaks. The first peaks are clearly generated by light-adapted cells, for their latencies are much shorter than those of the dark-adapted responses in the left column. (The responses in the left and middle columns were recorded on different time scales (see caption) so that comparisons of latencies by inspection are difficult. Comparisons of latencies will be easier in Fig. 10 where all responses were recorded on the same time scale.) The second peaks have latencies about the same as responses from dark-adapted eyes, which implies that the cells generating the second peaks are hardly light-adapted at all by the broad-band blue, yellow, or orange background lights. However, we have been able to light-adapt the second peaks using narrow-band lights in the wavelength ranges which elicit the second peaks. This is illustrated in the right column of Fig. 5. There, the eye was again light-adapted by the same amount as in the middle column, as determined at 500 nm, observing the first peak of the response. However, the adapting wavelength was now 615 nm. Because the cells generating the first peaks were equally light-adapted as before, the 620 and 640 nm flash intensities used were about the same as in the middle column and gave about the same size first peaks. However, these flashes now elicited much reduced second peaks. This is thus consistent with the conclusion that the cells generating the second peaks were now less sensitive because they were now being somewhat light-adapted too. Since the waveforms of the responses in the right column of Fig. 5 are now much like the responses to 500 nm flashes, all this is also consistent with the assumption that the second peaks are simply potentials adding on to the first peaks.

ORIGINS OF THE SECOND PEAKS   Only long wavelengths elicit these second peaks, which in this respect are like the "red receptors" in flies' eyes (Walther and Dodt, 1959). Red receptors are artifacts in intracellular recordings from flies' eyes (Burkhardt, 1962). Goldsmith (1965) attributes the increased red sensitivity of the fly ERG to recruitment of additional cells by light scattered through red-transparent shielding pigments (Strother, 1966). The second peaks here might arise analogously. In spider secondary eyes, the rhabdomes are separated by pigment sleeves, as mentioned earlier. When these eyes are looked into ophthalmoscopically, the pigments appear "light brown" (Salticidae, jumping spiders: Homann, 1928), "brick-red," "yellow," or "black" (Thomisidae, crab spiders: Homann, 1934), or "violet-rose" (Lycosidae, wolf spiders: Homann, 1931). We have seen the latter color in our wolf spiders too. Presumably, light scattered through these pigments could be the direct, incident light as well as that scattered upon reflection from the tapetum.

To test the scattered light, screening pigment hypothesis, spectral sensitivities were determined from the second peaks. The results are shown at the left in Fig. 6. The maximum sensitivity is at 580 nm, but the sensitivity below 580 nm is not known, because there were no second peaks elicited there with the intensities used. Assume now that the second peaks are due to (nearly)

dark-adapted cells. Then, the difference between the spectral sensitivities of the dark-adapted eye (Fig. 4) and of the second peaks is a measure of the density of material between the light source and these cells. Such differences are shown at the right in Fig. 6 (left ordinate). For comparison, the solid line is the unscaled spectral absorption of red screening pigment in the compound eye of the housefly *Musca* (Goldsmith, 1965; Strother, 1966). The fit is not good, and would not be improved by scaling. The shielding in the spider eye does not reach zero density at 660–680 nm, where the fly screening pigment does. Clearly, some other screening pigment would have to be involved in the spider posterior median eye.
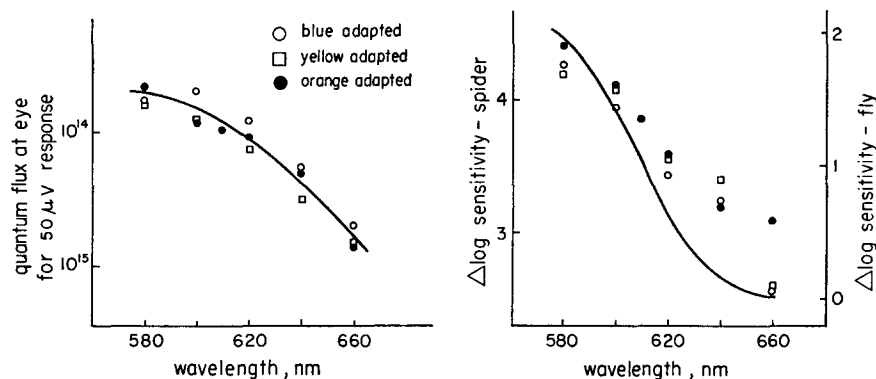

Figure 6. Spectral sensitivities of second peak responses. Left, the quantum fluxes needed to elicit 50 µv second peaks are shown on the ordinate vs. wavelength on the abscissa. Solid line drawn by eye is without theoretical significance. Right, the left ordinate gives the differences in log units between the fluxes required at each wavelength to elicit 50 µv peak responses from the dark-adapted eye or as second peak responses. The symbols used at the left apply here, too. The left ordinate gives directly the differences for the blue-adapted eye, while the points for the yellow- and orange-adapted eye have been slid down by 0.1 log unit. The solid line, whose values are given on the right ordinate, is a similar difference spectrum taken from Goldsmith (1965, Fig. 11) and represents the effect of a red screening pigment (Strother, 1966) in the fly's eye.

ANTERIOR AND POSTERIOR LATERAL EYES The other two pairs of secondary eyes, the anterior and posterior lateral eyes, have spectral sensitivities very much like those of the posterior median eyes. Fig. 7 illustrates their dark-adapted spectral sensitivities (along with the spectral sensitivity of an anterior median eye, to be discussed below). For these secondary eyes, 505 nm nomogram curves (thick lines) appeared to fit better than 510 nm curves. The difference is unexplained, but perhaps there was some systematic error involved in the different calibration methods used for the various fiber optics (see Methods). In the anterior lateral eye, the entire nomogram curve fit the data, but in the posterior lateral eye, the nomogram curve was broader at short wavelengths. The spectral sensitivity curves for the posterior lateral eye and for

the posterior median eyes previously illustrated are nearly the same, while the anterior lateral eye is slightly more sensitive in the blue and near-ultraviolet. We have done no experiments with light-adapted anterior and posterior lateral eyes, but we presume that they too would show no selective chromatic adaptations. That is, the similarities between spectral sensitivities of secondary eyes are so great (and so different from the spectral sensitivities of anterior median eyes, below), that it is likely that all secondary eyes contain only the one type of visual cell with maximum sensitivity at 505–510 nm.
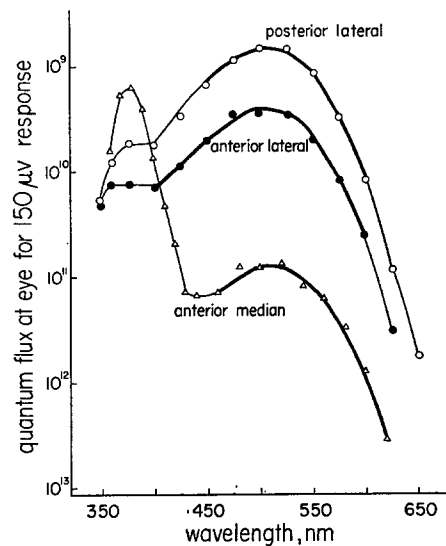

FIGURE 7.   Spectral sensitivities of dark-adapted principal and secondary eyes. Results were obtained in an initial experiment with wavelengths spaced 25 nm apart. To fill in the gaps in the spectral sensitivity curve of the anterior median eye, a second experiment was run on the same eye. It was now 0.15 log unit more sensitive in the blue-green and 0.1 log unit less sensitive in the ultraviolet. Since it is a better defined curve, it is shown here (as open triangles). The heavy lines on all three curves represent a 505 nomogram pigment, where it fits. Elsewhere, the nomogram curve was too broad, and thin lines were drawn freehand through the remaining points. In this and all experiments subsequently illustrated, a single fiber optic was used to stimulate the eye. *Lycosa baltimoriana.*

Principal Eyes (Hauptaugen)

DARK-ADAPTED EYES   The open triangles on Fig. 7 show the spectral sensitivity of an anterior median eye. Its absolute sensitivity is in correct proportion to those of the anterior and posterior lateral eyes. Thus, it is almost as sensitive in the ultraviolet at 380 nm as they are in the blue-green, but much less sensitive (-1.8 log units, or $\frac{1}{63}$rd) in the blue-green, where it has a secondary maximum at 505 nm. These results on this anterior median eye are more or less typical of (all but two) anterior median eyes studied.

As mentioned earlier, the enhanced relative ultraviolet sensitivity was expected from the preliminary experiments on anterior median eyes of jumping spiders (DeVoe and Zvargulis, 1967). A comparison can be made with *Limulus*, whose median eyes are of the same embryological origin (second cephalic lobe: Lambert, 1909) and also have enhanced ultraviolet sensitivities (Wald and Krainen, 1963; Chapman and Lall, 1967; cf. also Nolte, Brown, and Smith, 1968). On the other hand, the web-building spider *Tegenaria*, unlike the vagabond wolf and jumping spiders, does not appear to have enhanced ultraviolet sensitivity in its anterior median eyes (Giulio, 1962). Likewise, scorpion median eyes, homologous to spider anterior median eyes (Patten, 1889), have little or no ultraviolet sensitivity (Machan, 1968 *b*). Scorpion lateral eyes (homologous to spider secondary eyes), moreover, do have three to four times more sensitivity to ultraviolet than to visible wavelengths. Similarly, spider mites, *Tetranychus*, which have only lateral eyes (Hughes, 1959), are at least twice as attracted to ultraviolet as to visible wavelengths (Naegele, McEnroe, and Soans, 1966). Even among the arachnids, then, high ultraviolet sensitivities are not exclusive to median eyes, and they are found in pineal eyes of frogs (Dodt and Heerd, 1962) as well as in lateral compound eyes of owlflies *Ascalaphus* (Neuroptera) (Gogala, 1967).

VARIATIONS IN ULTRAVIOLET SENSITIVITIES   The next point we wish to make is that in two anterior median eyes from two wolf spiders, there were no enhanced ultraviolet sensitivities either. The results from one of the animals is shown at the top of Fig. 8. Not only was this eye of extreme absolute sensitivity (compare Figs. 7 and 8), but the maximum relative spectral sensitivity was at 505 nm as well. There was only a small submaximum in sensitivity at 380 nm.

With a flash duration of 45 msec, and with about 450 receptor cells per anterior median eye (Melamed and Trujillo-Cenoz, 1966), 50 μv responses required about 12 quanta/cell incident at the cornea. For a 20 μv "threshold" response, the number would have been about 5 quanta/cell incident at the cornea. The light losses from reflection and absorption in the spider eye are unknown, but even if they were taken into account, the threshold sensitivities would be unlikely to approach those of 1 quanta/200 rods observed in the albino rat (Cone, 1963).

When the same eye was tested 7 wk later, the absolute sensitivity at 500 nm had dropped 6 log units (lower curve, Fig. 8), and the maximum relative sensitivity was now at 370–380 nm. That is, the absolute sensitivity had not dropped as greatly at 380 nm as it had at 500 nm. The changes in relative spectral sensitivities can be better seen in Fig. 9, where the open symbols are the same as in Fig. 8. The closed symbols refer to two similar experiments 10 wk apart on the anterior median eyes of another spider. The absolute sensi-

tivities for all experiments are given in the inset in terms of the quantum fluxes at 500 nm needed to elicit 50 μv responses. In these two animals, then, relative ultraviolet sensitivities increased from 30 to 300 times between the first and second experiments, although absolute sensitivities at *all* wavelengths dropped precipitously.
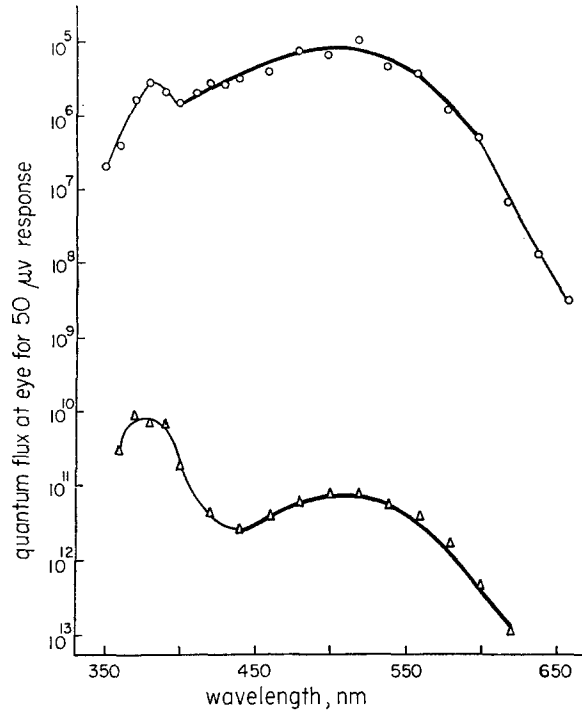

FIGURE 8.   Two absolute and spectral sensitivities from a left anterior median eye. The open circles were recorded in the first experiment on this eye, the open triangles from a second experiment 7 wk later. At the time of the second experiment, the right eye had a spectral sensitivity similar to that shown here for the left eye. The ordinate shows the quantum fluxes needed at each wavelength to elicit 50 μv peak responses. Thickened lines are 505 nm nomogram curves; thin lines were drawn freehand through the remaining points. Flash durations were 45 msec. *Lycosa baltimoriana.*

Such absolute and spectral sensitivity changes are the largest that we have seen, but they are by no means isolated incidents. Dark-adapted anterior median eyes have far more variable spectral sensitivities than do posterior median eyes. For example, the anterior median eye of the animal whose spectral sensitivities are illustrated in Fig. 7 first had an ultraviolet:visible sensitivity ratio of 2 log units, next 1.8 log units (illustrated in Fig. 7), then 1.35, 1.25, and finally 0.8 log units. We can give no firm statement as to what these variabilities in spectral sensitivities (as well as some to be illustrated below)

are due to, or even as to whether they have a common origin. We can, however, exclude changes in numbers of cells in these eyes. Unlike *Limulus* lateral eyes (Waterman, 1954), there are no increases in numbers of visual cells from molt to molt (Homann, 1931), and in any event, there were no intervening molts in any of our animals.
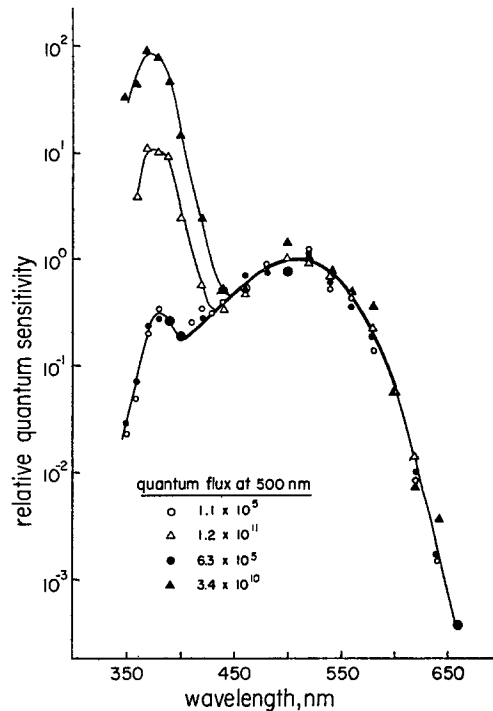

Figure 9. Anterior median eye spectral sensitivities with and without enhanced ultraviolet sensitivity. Results from four experiments on two animals. The thickened line is a 505 nm nomogram curve, the thin lines were drawn freehand through the remaining points. Relative quantum sensitivity is the reciprocal of the number of quanta/sec needed to elicit a 50 μv criterion response. The needed quantum fluxes at 500 nm are listed below the curves. Open circles and open triangles, results from Fig. 8. Filled circles and filled triangles, results from two experiments on the left and right anterior median eyes, respectively, of one *Lycosa miami* performed 10 wk apart. In both animals, the experiments shown by open and filled circles were performed first and within 5 days of each other.

WAVEFORMS OF RESPONSES FROM ANTERIOR MEDIAN EYES   Although the retinal elements are inverted in anterior median eyes, compared with the secondary eyes (Fig. 1), the ERG's are not. Light still elicits cornea-negative potentials, as it does from the secondary eyes. This can be seen in the responses illustrated in Fig. 10. In the ultraviolet-adapted eye (middle row), response waveforms are faster than in the dark-adapted eye, as is usual for

light-adapted eyes (DeVoe, 1967 a). In addition, there is a second peak in waveforms of responses to visual wavelengths (for example, 500 nm) but not to ultraviolet wavelengths (for example, 380 nm). The second peaks in ultraviolet-adapted, anterior median eyes are elicited by all wavelengths 400 nm and longer (see Fig. 11, open triangles). This is in contrast to the second peaks from light-adapted posterior median eyes, where the second peaks were
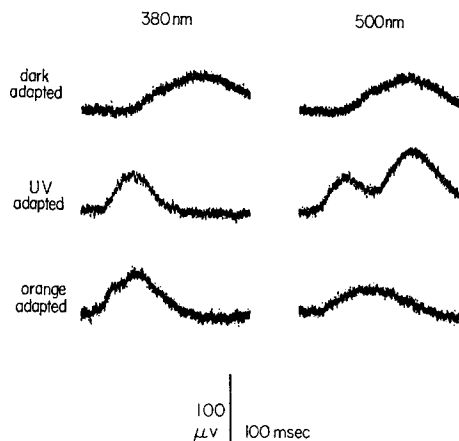

Figure 10. Waveforms of responses of a light- and dark-adapted anterior median eye Recorded responses to 380 and 500 nm flashes are shown for the dark-adapted, ultraviolet-adapted, and strongly orange-adapted eye. Upward deflection indicates corneal negativity with respect to an indifferent electrode. This anterior median eye had the enhanced ultraviolet sensitivies shown in Fig. 11. The 500 nm response of the ultraviolet-adapted eye has a distinct second peak, that of the orange-adapted eye does not. There are no second peaks in 380 nm responses. Ultraviolet adaptation used predominantly the 365 nm line from a Gates mercury arc lamp isolated by a Wratten 18A filter. Orange adaptation was provided by a Schott OG-550 filter (passes all wavelengths 550 nm and longer) in the tungsten beam. The calibration marks at the bottom apply to all records and tracings. *Lycosa carolinensis.*

elicited only by wavelengths 580 nm and longer. On the other hand, as in posterior median eyes, the second peaks in ultraviolet-adapted anterior median eyes have latencies about the same as the responses from dark-adapted eyes (compare the responses in the right column in Fig. 10). Also similarly, lights which elicit the second peaks adapt them away as well. In Fig. 10, third row, the second peak in the response to a 500 nm flash is partially light-adapted away by the orange background illumination and blends with the first peak. In another experiment, the 500 nm responses of orange-adapted eyes were identical to the first peaks of the same eyes when they were ultraviolet-adapted. This indicates that in the anterior median eyes too the second peaks may be elicited and adapted away independently of the first peaks.

SPECTRAL SENSITIVITIES OF CHROMATICALLY ADAPTED ANTERIOR MEDIAN EYES Fig. 11 shows the spectral sensitivities of the eye whose response waveforms are illustrated in Fig. 10. On the left, the absolute sensitivities are given, in terms of quanta/sec incident on the eye. On the right, relative spectral
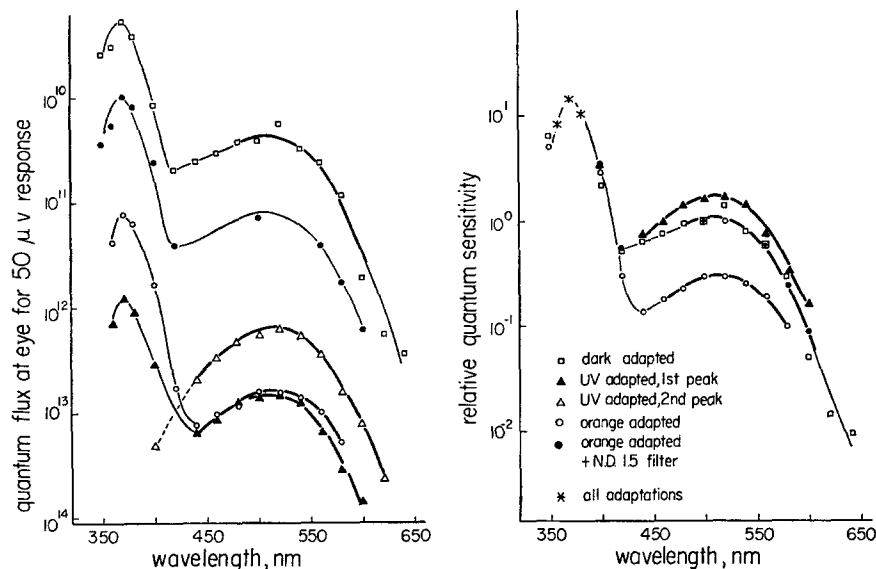

FIGURE 11. Spectral sensitivities of a light- and dark-adapted anterior median eye. Left, absolute sensitivies; right, relative sensitivities with all curves superimposed at 370 nm. In both parts of the figure, the thickened lines represent nomogram pigment curves with the following maxima, dark-adapted, first peak of ultraviolet-adapted, and weakly orange-adapted, 508 nm; second peak ultraviolet-adapted and strongly orange-adapted, 513 nm. Thin lines were drawn freehand to fit the remaining points. In the left figure, the thin line through the filled circles (weakly orange-adapted) is the curve above from the dark-adapted eye shifted down 0.73 log unit. Data represented by the filled circles were recorded 70 min and more after the start of weak orange adaptation (see Fig. 12). This is the only experiment in which the spectral sensitivity curve for the first peak responses of the ultraviolet-adapted eye did not match exactly the dark-adapted curve. Sources of adapting lights are given in the caption to Fig. 10. Optics Technology metal film, neutral density filters were used for the weak orange adaptation. *Lycosa carolinensis.*

sensitivities are given, with all curves made to coincide at 370 nm. Ultraviolet adaptation was the greatest here of any experiment, 2.7 log units, vs. 2.2 log units or so maximum reduction in sensitivity at 370 nm in other experiments. (In all these experiments, the full intensity of the ultraviolet-adapting beam was used.) Perhaps as a result, this was the only experiment in which ultraviolet adaptation was possibly selective: It reduced the 370 nm:500 nm sensitivity ratio, although only by 0.2 log unit (1.6 times). In all

other experiments, the spectral sensitivity curves of ultraviolet- and of dark-adapted eyes had identical shapes.

Likewise, in this and in most other experiments, the full intensity of the orange-adapting beam reduced the relative sensitivity at wavelengths 420–440 nm and longer. This can be seen at the right in Fig. 11 (open circles). Orange adaptation consistently shifts the maximum visible sensitivity a possibly insignificant 5 or so nm towards longer wavelengths (513 vs. 508 nm in Fig. 11). The reason for this is not known.

Chromatic adaptations of the very sensitive eyes (open and filled circles in Fig. 9) caused no changes in relative spectral sensitivities at all, even to orange adaptations. In this respect they behaved like the posterior median eyes. Unlike all other eyes, however, we never recorded second peaks in waveforms of responses from these very sensitive eyes. Two possible reasons come to mind. If, on the one hand, second peaks are due to light leakage through screening pigments, as has been argued earlier in this paper, perhaps such screening pigments were absent in these very sensitive eyes. Indeed, the absence of screening pigments might have been the reason for the very high sensitivity of these eyes. Goldsmith (1965) has pointed out that mutant white-eye flies which lack red screening pigments not only lack enhanced red sensitivity, but also are about 2.6 log units more sensitive than wild-type flies. However, we have no confirming histological sections of the very sensitive anterior median spider eyes, and in any event, the shielding pigments in the more normal, enhanced ultraviolet-sensitive eyes do not appear to lie between the rhabdomes anyway (Baccetti and Bedini, 1964. Histological sections of some of our spiders, including the one used for the results in Figs. 10 and 11, confirm these authors.) The other alternative is that screening pigments did exist in the very sensitive eyes, but since these eyes were so sensitive, the relatively dim flashes which were adequate to elicit responses from directly illuminated cells were not adequate when they passed through the screening pigments to stimulate shielded cells.

POSSIBLE RETINOMOTOR ACTIVITIES IN ANTERIOR MEDIAN EYES   The depression of sensitivity at visible wavelengths by strong orange adaptation (and by only 0.6 log unit at that) illustrated in Fig. 11 is not necessarily due to selective adaptation of a visible color mechanism. Instead, we now present evidence that it might be due to retinomotor (i.e., nonreceptor) effects. After strong orange adaptation of the eye used for the results in Fig. 11, the orange light was reduced in intensity by 1.5 log units. The final spectral sensitivity of this eye, weakly orange-adapted, is given in Fig. 11 by the filled circles and is the same, relatively, as that of this eye dark-adapted (see the open squares and the filled circles in the right-hand part of Fig. 11). These results were obtained after 70 or more min under the weak adaptation. We initially began measuring the spectral sensitivity after only 30 min of weak orange adaptation, as we

assumed that this would be sufficient time for the receptor cells to adapt to the lower background illumination. These initial results, obtained between 30 and 70 min after reducing the intensity of the orange adapting light, are shown in Fig. 12 at the right as open circles. There is considerable scatter in the data, but the initial spectral sensitivity in the visible region (at wavelengths 420 nm
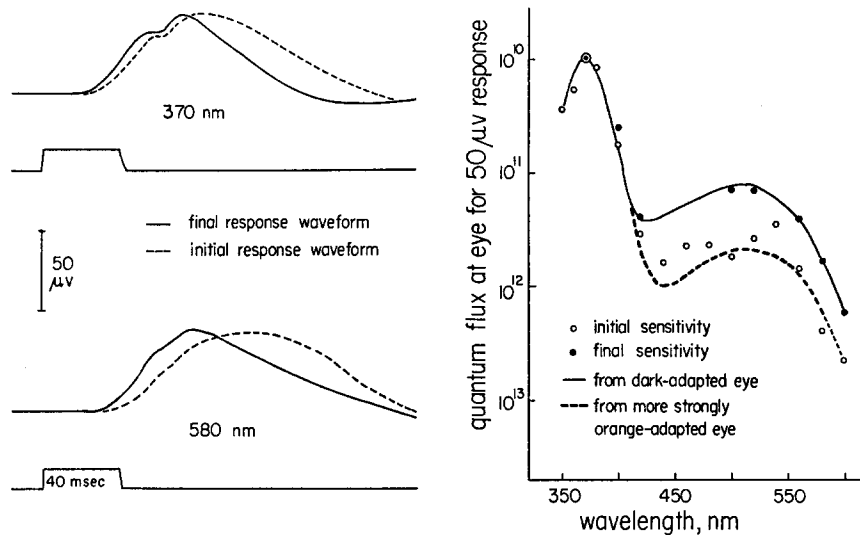

FIGURE 12. Changes with time in spectral sensitivity and response waveforms from a weakly orange-adapted anterior median eye. This is the same eye, weakly orange-adapted, as in Fig. 11. The open circles on the right and the dashed response tracings on the left were taken beginning 30 min after the placement of neutral density filters in the orange-adapting beam (strong to weak orange adaptation). The closed circles on the right (and the solid tracings on the left) were taken beginning 70 min after the start of weak orange adaptation and are the same as the filled circles shown in Fig. 11. The solid and dashed lines at the right are taken from Fig. 11, where they were used to fit the spectral sensitivities of the dark-adapted and strongly orange-adapted eye, respectively. Responses at the left are about 50 μv each. *Lycosa carolinensis.*

and longer) was low and most like that of the strongly orange-adapted eye. The curve traced through the spectral sensitivity of the strongly orange-adapted eye in Fig. 11 is here given by the dotted line. Below 420 nm, there was little difference between the initial and final sensitivities.

Coincident with increases in final, visible wavelength sensitivity came changes in waveforms of response. Had the increases in sensitivity been due to further receptor cell adaptation, the final waveforms would have been slower (Fuortes and Hodgkin, 1964; DeVoe, 1967 *a*). On the contrary, the waveforms became faster. This is shown on the left in Fig. 12, where we have compared the responses recorded both before and after the changes in visual wavelength

sensitivity. It seems likely that the increases in speeds of response occurred because the cells became more light-adapted, although the background illumination was unchanged all this time. The cells could have become more light-adapted if shielding pigments or other structures moved and allowed more of the adapting light to reach the cells. In turn, more of the test lights would also have reached the cells. The results in Fig. 12 would then be explicable if the decreases in sensitivity due to increased light adaptation were more than counterbalanced by the greater intensities of test flashes reaching the cells.

We mention shielding pigments because Scheuring (1914) has claimed that these may move distally in the anterior median eyes of wolf spiders placed in sunlight, even to the point of covering the distal ends of the receptor cells. As mentioned above, the shielding pigments in anterior median eyes appear to be retracted behind the rhabdomes. It therefore seemed possible that small distal movements of shielding pigments during our experiments might have changed the amounts of light reaching the cells and caused the results pictured in Fig. 12. Presumably, such distal pigment migrations would be elicited by orange lights, but not by ultraviolet adapting lights.

To test the above retinomotor hypothesis, we adapted the anterior median eyes of one animal each for 2 hr to the dark, to ultraviolet light, and to orange light. The animals were then cut in half, fixed overnight in Bouin's solution, dehydrated, double-embedded in celloidin-paraffin, and mounted unstained after sectioning (Machan, 1966). However, we could then see no observable differences between pigment positions in any of the three animals, much less the full distal migration which Scheuring (1914) claimed to have observed. Possibly the lights were not bright enough, although they were the same lights that were used in the experiments illustrated in this paper. Possibly too there were pigment migrations, which could only be determined from more extensive and quantitative measurements. In any event, there were no striking differences in pigment position between orange- and ultraviolet-adapted eyes that would explain the results in Fig. 12. What retinomotor activity, if any, could have caused those results remains unknown.

DISCUSSION

The results on spectral sensitivities of wolf spider eyes may be summarized as follows: chromatic light adaptations of posterior median eyes (secondary eyes) never changed relative spectral sensitivities of directly illuminated cells, even when these light adaptations reduced absolute sensitivities by up to 4.7 log units (to $\frac{1}{50,000}$th). Posterior median eyes therefore probably contain a homogeneous population of receptor cells all having a visual pigment absorbing maximally at 505–510 nm. The other secondary eyes (anterior and

posterior lateral eyes) when dark-adapted have spectral sensitivities very similar to those of posterior median eyes and therefore most likely also contain only this same visual pigment in all visual cells.

We conclude tentatively that anterior median eyes likewise have homogeneous receptor populations with the same pigment *systems* in all cells. Chromatic adaptations of up to 2.7 log units (reduction in sensitivity to as little as $\frac{1}{500}$th) had only meagerly selective effects on relative spectral sensitivities (Fig. 11), and some of these "selective" adaptations may have been due to retinomotor activities at that (Fig. 12). Therefore, it appears that all adapting lights reduced the sensitivities of all cells just about equally, at least with the intensities of adapting lights of which our equipment was capable. This is to say that there were few differences between spectral properties of cells in a given anterior median eye in a given experiment.

Enhanced Ultraviolet Sensitivities of Anterior Median Eyes   One striking result of all our experiments on all wolf spider eyes, the anterior median eyes included, is that the visible wavelength portions of all spectral sensitivity curves have had maxima in the region 505–510 nm. The anterior median eyes alone have had enhanced ultraviolet sensitivities. From the evidence above, this additional ultraviolet sensitivity must be a property of the same cells that are sensitive at 505–510 nm as well. One possibility, then, is that there is only one *visual* pigment in anterior median eye visual cells, but that there is in addition an ultraviolet-absorbing substance in these cells which fluoresces at visible wavelengths. The fluorescence in turn would be absorbed by the visual pigment to excite the cells (Chance, 1964).

We tested the above possibility as follows: a spider was anesthetized, its abdomen and legs were cut off, and it was waxed upside down to a cork block which was placed in spider Ringer (Rathmeyer, 1965). Next, the chelicerae (*Ch* in Fig. 1 A) were cut off at the level of the articulation membrane (*Mb* in Fig. 1 A). This exposes the anterior median eyes from below (compare Fig. 1 A and 1 B). Then, a fiber optic was brought up to one of the anterior median eyes, and the eye was illuminated by either ultraviolet light (365 nm) or blue-green light (500 nm). Under visual observation, the blue-green light could be seen to emerge distinctly from the back of the illuminated eye, especially when the pigment sheath around this eye was gently torn. No light whatsoever was seen emerging with ultraviolet illumination of the eye. The blue-green and ultraviolet lights were, however, approximately matched for equal stimulating effect, as determined with microelectrode recordings from this eye. Moreover, this eye was about 20 times more sensitive to the ultraviolet light, so it was clear that we were dealing with an eye with enhanced ultraviolet sensitivity. Since the ultraviolet and blue-green lights were matched for equal stimulating effect, any fluorescence would have been at least as bright as the blue-green

light and should have been visible. Since no fluorescence was visible at all, we conclude that fluorescence cannot account for ultraviolet sensitivities of anterior median eyes, much less their enhanced ultraviolet sensitivities.

Other possible ways of obtaining high ultraviolet sensitivities might be radiationless energy transfer from an ultraviolet-absorbing accessory pigment, or two visual pigments in one cell. In blue-green algae, radiationless transfer of absorbed energy from phycocyanin to chlorophyll may be as efficient as 95 % (Blinks, 1964). If such energy transfer occurred in spider anterior median eyes, fluorescence under ultraviolet illumination might not be observed. Yet, there could still be only one visual pigment for visual excitation. However, this visual pigment should bleach under strong light, whereas the accessory pigment most likely would not. Thus, an accessory pigment could be looked for in isolated visual cells with a microspectrophotometer.

Alternatively, there might be two visual pigments in visual cells of anterior median eyes, with a common pathway by which their breakdown products could excite the cell (Bennett et al., 1967. Two pigments would easily fit into a model developed for spider eye dynamics. DeVoe, 1967 b, Fig. 11.) That is, a cell light-adapted by selective adaptation of one pigment would be light-adapted and hence equally insensitive to excitatory contributions from the second pigment as well. If there were two such pigments equally coupled to excitation of the visual cells, they could not be resolved by studying excitation alone, as we have been doing here. On the other hand, both pigments should bleach under strong light. Therefore the two visual pigment hypothesis might be distinguishable from the accessory pigment, one visual pigment hypothesis on the basis of spectrophotometry of single visual cells. This remains to be attempted.

Second Peaks in Waveforms of Responses of Light-Adapted Eyes   The presentation above of second peaks in waveforms of response has sought to show that second peaks were generated by cells unadapted by the background illuminations used. The presumption was that these cells were protected from the background illuminations by screening pigments or structures. However, an alternative view might be that these cells were directly illuminated by the background illuminations, but were not adapted by them. That is, these cells had different spectral sensitivities than the majority of the cells in an eye.

Second peaks in responses from light-adapted posterior median eyes, at least, are unlikely to be coming from cells which are directly illuminated. This is because these cells would have a maximum spectral sensitivity somewhat below 580 nm (cf. Fig. 6) and would surely have been adapted strongly by the broadband orange background illumination. (The orange adapting light consisted of all wavelengths over 550 nm from a tungsten lamp.) However, the independent receptor hypothesis cannot be completely ruled out for the anterior median eyes. Basically, it can be argued that while most of the cells in

these eyes are alike in their spectral characteristics, a very few cells are different (say, 1 to 2 %) and do not respond at all to wavelengths less than 400 nm (cf. Figs. 10 and 11). Then, reduction of the sensitivity of all the other cells to $\frac{1}{100}$th or so by selective adaption would unveil the few, unadapted, different cells. For example, the sensitivity at 500 nm of second peaks in an anterior median eye was about $\frac{1}{80}$th that at 500 nm of the sensitivity of the dark-adapted eye (Fig. 11), and the spectral sensitivity of the second peaks was well fit by a 513 nm nomogram pigment (heavy line in Fig. 11). Clearly, if such different cells existed, the only way they would differ from the majority of cells would be that they were insensitive to ultraviolet lights. In the visible region of the spectrum, all cells in anterior median eyes would have the same spectral sensitivities. However, the existence of even a few, different cells would be a basis for color vision.

That there might be directly illuminated cells insensitive to ultraviolet lights seems improbable, however, if only because of the likelihood of ultraviolet β-band absorption in visual pigments. Such cells would have to contain a strongly ultraviolet-screening substance, which reintroduces the probability that screening pigments are involved in the origins of second peaks. Rather, there is an alternative model for the generation of second peaks in waveforms of response which has the same basis in all spider eyes and which does not involve assumption of more than one type of receptor cell in any one eye. First of all, it can be argued that second peaks do not even originate in the eye that is being illuminated. In such an eye, the adapting background illumination from the rather spatially restricted fiber optic illuminates only a portion of the retina, while the unilluminated cells are protected from scattered light by shielding pigments (in secondary eyes, at least). However, cells closest to the illuminated area should be exposed to the brightest scattered light through the least thicknesses of shielding pigments. Hence these close-in cells should be more light-adapted than cells farthest from the illuminated area of the retina, although less light-adapted than directly illuminated cells. In sum, there should be a dispersion of the second peak latencies, corresponding to dispersion in degree of light adaptation. The second peaks, however, are never "smeared out" in time, not even in the anterior median eyes, where the shielding pigments do not appear to lie between the rhabdomes and so can hardly shield unilluminated cells from stray light at all.

The alternative, then, is that second peaks in waveforms of responses come from other eyes. Wolf spider eyes are crowded together on the dorsum of the animal (see Fig. 1 A), and histological sections (such as illustrated in Fig. 1 B) show there to be ample opportunity for light leaking out the back of one eye to enter the back of another. With this alternative, we can explain the spectral differences between second peaks in waveforms of responses from principal eyes and from secondary eyes. Assume that both eyes contain pigments which

strongly absorb ultraviolet wavelengths below 400 nm or so and which are neutral attenuators of wavelengths above 400 nm. The second peaks from principal eyes are then responses from other eyes to lights with the ultraviolet filtered out. The secondary eyes, however, contain a reflecting tapetum (T in Fig. 1 C), whereas the principal eyes have, at most, scattered tapetal-like crystals (Baccetti and Bedini, 1964. We have never seen such crystals in sections of anterior median eyes from our animals, however. Illumination of the sections with incident light or through crossed polarizers brings out the tapetal crystals in secondary eyes (Homann, 1955), but has never revealed any such crystals in the principal eyes.) The layered tapetal structure in wolf spider eyes (Melamed and Trujillo-Cenoz, 1966) is similar to the structure of the reflecting argentea in the scallop, *Pectin* (Land, 1966), and like the argentea might also be a "multilayer interference reflector." Tapetal reflections appear "green" in wolf spider secondary eyes (Homann, 1931). Red lights outside the green reflection band would then be transmitted instead of reflected. Therefore, the sudden appearance of second peaks in secondary eye waveforms of response at wavelengths 580 nm and longer may simply result from sharp transmission-reflection characteristics of tapeta. That is, from the structure of secondary eyes in Fig. 1 C, only light which passes unabsorbed down the rhabdomes is reflected or transmitted at the tapetum, while all other light encounters the absorbing pigments between rhabdomes. In this way, only long wavelengths would leak out of secondary eyes in sufficient intensity to stimulate other eyes. All wavelengths longer than 400 nm would leak out of principal eyes. The virtue of this model, then, is that second peaks in waveforms of responses from all light-adapted wolf spider eyes have the same origins, and it is unnecessary to propose one origin for second peaks from the principal eyes and another for those from the secondary eyes.

We made one attempt to test the above model. We arranged to record from one posterior median eye in the usual way, then placed half a ping-pong ball over the whole animal. After light-adapting the selected posterior median eye through the fiber optic, we illuminated the whole of the ping-pong ball with white light from a microscope illuminator, in order to light-adapt all other eyes as well. Unfortunately, this procedure always further light-adapted the test eye, too, and presumably light-adapted some of the cells in the test eye which were not being directly illuminated by the light from the fiber optic. As a result, we could not verify where second peaks in waveforms of responses were coming from.

Absolute and Spectral Sensitivities of Anterior Median Eyes   Although we conclude that only one (major) spectral type of cell exists in each spider eye, the principal eyes did have two extreme forms of spectral sensitivities. We found high blue-green sensitivity coupled with high absolute sensitivity and high

ultraviolet sensitivity coupled with low absolute sensitivity. It appears that what controls absolute sensitivities may control spectral sensitivities as well.

Absolute sensitivity increases of up to 3 log units have been correlated with shielding pigment movements in dark-adapted moth eyes (Bernhard and Ottoson, 1964; Post and Goldsmith, 1965) and in dark-adapted median eyes in scorpions (Machan, 1968 a). However, these increases in absolute sensitivity in median eyes of scorpions were accompanied by increases in relative spectral sensitivity at near ultraviolet wavelengths (404 nm) (Machan, 1968 b) but not in the blue-green as in the spider. Moreover, we have already discussed the possibilities that shielding pigment movements in anterior median eyes might cause changes in relative spectral sensitivities of orange-adapted eyes (cf. Figs. 11 and 12) or of very sensitive anterior median eyes and have found the evidence wanting.

More certainly, the changes in spectral and absolute sensitivities are not due to diurnal rhythms of the type seen in beetles, *Dytiscus* (Jahn and Wulff, 1943), where diurnal sensitivity changes of up to 3 log units occurred. These changes were uncorrelated with shielding pigment movements (Jahn and Wulff, 1941) and therefore presumably had their origins in the receptor cells. However, spectral sensitivities were the same in both "day" and "night" eyes (Jahn and Wulff, 1948), but the ERG's changed electrical sign (Jahn and Wulff, 1943). These results on beetles are unlikely to apply to spider eyes, first, because all our experiments were done at the same time of day, second, because there were only minimal changes in waveforms of responses between sensitive and insensitive anterior median eyes, and finally, because spider spectral sensitivities *were* different between the two states of absolute sensitivity.

A number of further possibilities come to mind which might explain the observed changes in absolute and spectral sensitivities of anterior median eyes, but they are at best speculative. The main observation is that most of the differential effects of light adaptation (Figs. 11 and 12) and most of the striking changes in relative sensitivities (Fig. 9) occur either above or below 420–440 nm. If there were two visual pigments in each visual cell, the above variations around 420–440 nm might represent changes in couplings of the two pigments to excitation. However, changes in couplings would not explain the large changes in absolute sensitivities as well. Alternatively, suppose that in the 7–10 wk intervening between the experiments illustrated in Fig. 9, a large amount of screening substance was laid down in the rhabdomes of these eyes (and was there already in all other anterior median eyes tested). Many of our results could then be explained if this screening substance (a) were an accessory pigment transferring energy of ultraviolet quanta radiationlessly to the visual pigment, (b) absorbed neutrally (by up to 6 log units) at wavelengths 420 nm or so and longer, and (c) absorbed still more strongly at wavelengths shorter than 420 nm, thus preventing ultraviolet lights from passing through the eye.

Then, changes in the amount and position of, and effectiveness of the proposed radiationless energy transfer by, such an accessory pigment might account for all the observed variabilities in spectral sensitivities of anterior median eyes. Such an accessory pigment would presumably appear yellow, but if it exists, it does not remain in the visual cells during the fixation and embedding procedures we have used on our spiders. It might be visible in frozen sections, but we have not yet attempted to make these. Still another possibility for the large changes in absolute sensitivities in Figs. 8 and 9 is that the cells went into more or less permanent states of light adaptation, in which breakdown products of the visual pigment(s) remained in the eyes. Breakdown products like retinal would be yellow, and hence might serve the function of the accessory pigment above which filters out ultraviolet wavelengths. Reduction of absolute sensitivities would, on the other hand, be due not to filtering of lights by a pigment with a density of 6 (in the visible wavelengths), but would be due to the same processes whereby breakdown products from real light stimuli light-adapt the eye (DeVoe, 1967 b). It is not very clear, however, how the enhanced ultraviolet sensitivities of anterior median eyes would be explained by this scheme, unless ultraviolet energy absorbed in yellow breakdown products were to be passed on to a visual pigment, whereas visible wavelength energies were not. Clearly, there are many possible permutations and combinations of the above multiple visual pigment, accessory pigment, permanent light adaptation hypotheses, and further speculation does not seem justified at this point.

Color Vision in Spiders   There remains the question that originally set off this investigation: do the experiments of Kästner (1950) show that spiders can see color with their anterior median eyes (or with any other eyes?). On the basis of our results, spiders cannot, at least not with their anterior median eyes alone. It may be objected that Kästner's behavioral experiments were done with jumping spiders, whereas our electrophysiological experiments used wolf spiders. However, the preliminary spectral sensitivities recorded from anterior median eyes of jumping spiders in this laboratory (DeVoe and Zvargulis, 1967) appear much like the more extensive results on wolf spiders reported here. An example is given in Fig. 13. The main difference between wolf and jumping spiders is that the jumping spiders have a maximum sensitivity in the visible wavelength region at 530 nm, instead of at 505 nm as for the wolf spiders. Otherwise, as in the wolf spiders, there is an abrupt rise in sensitivity below 420 nm (which we could not fully measure in the ultraviolet with the tungsten light source available at that time), and chromatic light adaptations of 1 log unit (the amount used for the results in Fig. 13) do not change relative spectral sensitivities. It would therefore appear that jumping spiders also lack two or more, spectrally different, cell populations in their anterior median eyes.

There are two obvious explanations for Kästner's (1950) behavioral ob-

servations, however. One (mentioned in the introduction) is that the orange (or blue) colored stripes which the spiders always distinguished readily from all gray stripes always gave high contrasts to the gray stripes at ultraviolet wavelengths. Kästner tried to control for ultraviolet sensitivity by placing an
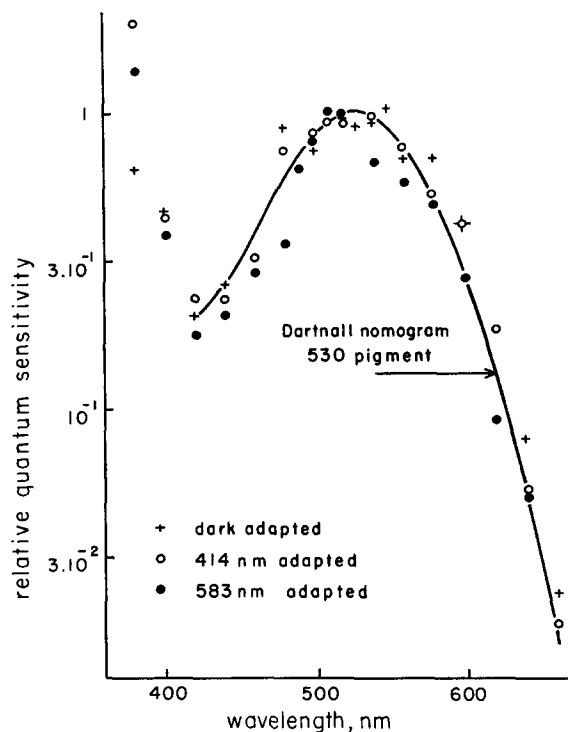

FIGURE 13. Relative spectral sensitivities of a dark- and light-adapted anterior median eye from a jumping spider. *Phidippus audax*. Ordinate, relative intensities, as quanta/sec required at each wavelength to elicit 50 μv responses. All curves have been normalized to a relative sensitivity of 1 at 530 nm. The solid line is a 530 nm nomogram curve (Dartnall, 1953). The absolute fluxes in quanta/sec required to elicit the 50 μv responses at 530 nm are, dark-adapted eye, 6.3 $\times$ 10^{10}; 414 nm and 583 nm adapted eyes, 6.6 $\times$ 10^{11}. These experiments were performed using an American Optical Co. LGM fiber optic bundle and a quartz-iodide tungsten lamp in the monochromator. Chromatic adapting lights were obtained using Optics Technology Monopass interference filters.

unfed spider and a fly in a box covered with an ultraviolet-transmitting filter and illuminating the filter with a mercury arc lamp. Only 2 of 34 spiders caught the fly under ultraviolet illumination, but 19 subsequently caught or jumped at the fly when the filter was removed and daylight was admitted to the box. Kästner concluded that his jumping spiders could not see the ultraviolet light. However, jumping spiders appear to use their wide-visual field, secondary eyes for the initial detection of prey, rather than their narrow-visual

field, anterior median eyes (Homann, 1928). Therefore, Kästner's experiment may show only that jumping spider secondary eyes, like those of wolf spiders, lack ultraviolet sensitivity.

A second possible explanation of Kästner's results arises from behavioral observations on jumping spiders by Dzimirski (1959). She found that animals with all but the anterior lateral (secondary) eyes covered would still react to striped walls like those used in Kästner's (1950) experiments. Thus, Kästner's animals may have been using two sets of eyes in their discrimination of colored stripes from gray stripes. That is, the differences in spectral sensitivities between principal and secondary eyes could form the basis of color vision. Thus, comparing the spectral sensitivities and absolute sensitivities of anterior median and lateral eyes in Fig. 7, ultraviolet wavelengths would appear bright to the anterior median eyes, but dim to anterior lateral eyes, while the reverse would be true for blue-green wavelengths. What remains to be proven is that the spider does or can make simultaneous use of the response from more than one set of eyes for the discrimination of color.

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