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VISION IN THE CTENID SPIDER *CUPIENNIUS SALEI*: SPECTRAL RANGE AND ABSOLUTE SENSITIVITY

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Summary

Electroretinograms were recorded from all eyes of the wandering spider *Cupiennius salei* (Ctenidae) and were found to be simple cornea-negative potential differences with amplitudes of up to 10mV. In both the principal eyes and all of the secondary eyes, the spectral response curves show a prominent green peak at 520 or 540nm and a shoulder in the ultraviolet between 340 and 380nm. The largest response in the ultraviolet measures between 65% and 80% of the green peak. Selective chromatic adaptation to either green or ultraviolet monochromatic light does not change these relative response levels and fails to indicate the presence of more than one spectral type of receptor. In the range 450–500nm, however, the Dartnall curve clearly deviates from the spectral sensitivity (SS) curve. Since the SS curves of all eyes have a small shoulder in the blue at 480nm, the existence of two or even three visual pigments is a possibility. Intensity curves were determined with white and monochromatic light. For white light, absolute corneal illuminance thresholds were clearly below 0.01lx. For monochromatic light stimuli, a corneal illuminance of approximately 3×10^{12} photonscm^{-2}s^{-1} is needed to elicit a half-maximal response. At threshold, the equivalent value is 3×10^{9} photonscm^{-2}s^{-1}, which corresponds to a retinal illuminance of 5.9×10^{9} photonscm^{-2}s^{-1}. Consequently, *Cupiennius salei* should be able to use its visual sense not only shortly after sundown but also under much poorer light conditions, such as those provided by moonlight. The log–linear response range of all eyes covers a stimulus intensity range of 4logunits. The sensitivity of the principal eyes increases by up to 0.81logunits at night as compared with daytime. The chromophore of the visual pigment of all eyes is 11-*cis* retinal.

Introduction

Mainly from the classical work of Homann (1928, 1931, 1951, 1971) and from the landmark studies of Land (1985) and Blest (1985), we know that spider eyes are far from being simple or having uniform properties throughout the taxon. There are masterpieces

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of eye design among the jumping spiders (Salticidae) and dinopids (Dinopidae). It is mostly these cases that are quoted as examples of perfection: a spatial resolution of 2.4′ in the anterior median eye of *Portia* (Salticidae; Williams and McIntyre, 1980); the light sensitivity of the posterior median eyes of *Dinopis*, which reach half of their maximum response at corneal fluxes corresponding to an intensity halfway between those of starlight and moonlight (Laughlin *et al.* 1980) and have a lens with an F-number of 0.58 (Blest and Land, 1977); or the tiered retina in the anterior median eyes of jumping spiders, which potentially serve to deal with chromatic aberration by being located in the best image planes for light of different wavelengths (Land, 1969*a*, 1985). Another fascinating aspect of spider eyes, in particular those of jumping spiders, is that the retinae of the anterior median or main eyes are moved relative to the lens to scan the image produced by the lens (Land, 1969*b*).

Eyes with such impressive properties and forming good images all belong to hunting spiders, such as salticids, lycosids, thomisids and sparassids. Orb weavers, in contrast, are generally considered to have comparatively poor vision with poor spatial resolution and with the image often out of focus at the photoreceptor level (Land, 1985; Yamashita, 1985).

In this paper we examine vision in *Cupiennius salei*, a ctenid that is closely related both taxonomically and behaviourally to the lycosids. *Cupiennius salei* is a nocturnal hunting spider and has been the subject of neurobiological research for a long time. Much of its behaviour, for example prey capture and courtship, has been shown to be guided predominantly by mechanosensory systems (Barth, 1985*a*,*b*, 1990; Barth and Schmitt, 1991; Seyfarth, 1985; Eckweiler and Seyfarth, 1988). This seems to make sense in a nocturnal species, whose members are active when very little light is available (Barth and Seyfarth, 1979; Seyfarth, 1980; Schmitt *et al.* 1990). There are several reasons, however, for taking a closer look at the visual capabilities of *Cupiennius*. One of these is that the optics of the eyes of this spider have recently been demonstrated to be of very good quality, with the F-numbers of the lenses being between 0.58 and 0.74, which suggests bright images and a reasonably well developed spatial resolution with angular separation of the receptor cells between 0.9° and 3.6° (along the rows they form). The impression of rather well-developed vision is further strengthened by neuroanatomical studies on the visual neuropiles in the central nervous system, first analyzed in spiders in *Cupiennius salei* in some detail (Strausfeld and Barth, 1993; Strausfeld *et al.* 1993). Finally, one of the similarities between the Ctenidae, to which the genus *Cupiennius* belongs (Lachmuth *et al.* 1985), and the Lycosidae, is their eye structure (Homann, 1961, 1966, 1971; Land and Barth, 1992). Since at least some of the lycosids have good vision (Land, 1985), a similar capacity may be present in *Cupiennius*.

With our previous neuroethological work on *Cupiennius* in mind, and following the idea of relating its visual capabilities to its behaviour, the prime objective of the present study is not so much visual physiology as such, nor the discovery of adaptations as sensational as those quoted for salticids and dinopids. Instead, we strive to determine whether *C. salei* is likely to see at night in the field and how much its four pairs of eyes differ in this regard. At the present early stage of this analysis, measurements of the electroretinogram (ERG) seemed to be both appropriate and sufficient.

Materials and methods

Animals

Adult females and a few males of *Cupiennius salei* Keyserling from our laboratory stock in Vienna were taken to Japan and kept in climatized boxes either under natural daylight conditions or in artificial light:dark cycles (12h:12h) at 25˚C. When kept under artificial light conditions (illuminance in the spider jars approximately 500–1100lx), the spiders were used for an experiment not earlier than 3 weeks after having been first exposed to these conditions. As shown by Seyfarth (1980), a few days suffice to change the activity rhythm of the animals according to the new L:D cycle. When comparing the responses of the spider in its subjective day and night (see Fig. 7), the animal in its day state was kept in the dark for 30min before the measurements to ensure dark adaptation.

Stimulation

A xenon arc lamp (500W, Ushio UXL 500 D-0) was used as a source of white light ranging in wavelength from ultraviolet to infrared. Quartz lenses formed a parallel beam of light, which passed, in the case of monochromatic stimuli, through one of 22 narrow-band interference colour filters (Vacuum Optics Corp., Japan, IF-S) covering the wavelength range between 290nm and 700nm. With the aid of a quartz neutral density filter (optical wedge), the various monochromatic lights were adjusted to contain an equal number of photons.

The end of the quartz light guide that led the light to the preparation was mounted on a manipulator which allowed the light source to be positioned in the middle of the visual field of the eye under examination (Land and Barth, 1992). For stimulation with *monochromatic light*, the light-emitting end of the light guide (diameter 3mm) was 3mm away from the eye surface. The maximum intensity available ($\log I=0$) was 3.11×10^{14} photons$\mathrm{cm}^{-2}\mathrm{s}^{-1}$ as determined with a radiometer (K.K. Sanso S.S., model 4700). In experiments with *white light*, the distance of the light guide from the eye was 100mm. This guaranteed a patch of light large enough to be measured with a digital illuminance meter (Topcon IM-3, Tokyo Optical Co. Ltd). The maximum intensity (illuminance) available in our experiments ($\log I=0$) was 900lx.

Stimulus duration was 15ms. The interval between stimuli was 20s in the case of spectral response measurements and intensity curve measurements. The precise timing of an experiment was controlled by using a computer.

Recording

ERG responses were recorded by placing the tip of a glass microelectrode (inner diameter of tip 78.3±20.6 μm, S.D.; resistance 0.55±0.83 MΩ, S.D.; $N=10$) very close to the eye's surface so that the electrolyte bridged the small gap between the electrode and the lens and provided electrical contact. Polyvinylpyrolidone was added to the electrolyte (spider or butterfly Ringer's solution, with no difference in recording quality) in order to reduce crystallization. The indifferent electrode was a silver wire slightly inserted into the opisthosoma of the spider. Conventional equipment was used to amplify, measure and record the ERGs.

Although the recording procedure of an ERG as such is straightforward, a particular problem in this case is the spider's tendency to move its prosomal tergum with its powerful musculature. These movements result in a change of distance between the electrode and the eye. To circumvent this problem, the legs of the animal were tightly fixed to the support with adhesive tape and, in addition, the prosoma was embedded in dental wax and its tergum firmly glued to a rod attached to a mechanically separate manipulator. The spiders survived the experiments and could be used repeatedly.

In some of our graphs variation is given as S.E. of the mean. We use N and N' to indicate the numbers of the spiders and eyes, respectively, used for an experiment and n to indicate the number of measurements.

Photopigment

The chromophore of photopigment contained in the eyes was identified using HPLC and following the methods described by Suzuki and Makino-Tasaka (1983). Chromophores extracted from the crayfish eyes (*Procambarus clarki*) were used as standards for 3-dehydroretinoids, chromophores extracted from the eye of *Papilio* as a standard for 3-hydroxyretinoid (Seki *et al.* 1987). A standard for retinoids was available commercially (Sigma).

Ultraviolet photographs

Photographs of male and female spiders of *Cupiennius salei* and *C. coccineus* were taken using the following material: film, Kodak Tri-x; lens, 50% transmittance at 360nm and 81% transmittance at 380nm; filter, Kodak 18A, 320–390nm.

Results

Like most spiders, *Cupiennius salei* has four pairs of eyes. These are called the anterior median (AM), anterior lateral (AL), posterior median (PM) and posterior lateral (PL) eyes (Fig. 1). Features characteristic of the genus and most valuable for quick identification in the field are (i) the specific arrangement of the eyes on the prosoma, (ii) the round circumference of the lenses of *all* eyes and (iii) a size relationship between the lens diameters of AM:AL:PM:PL eyes of roughly 1:0.5:1.5–2:1.5–2 (Lachmuth *et al.* 1985).

The ERGs recorded from all of the eyes are simple cornea-negative waves (Fig. 1) very similar to the monophasic ERGs long known for many arthropods (Autrum, 1958). Such ERGs are generally considered to represent responses of the retina only, not of the optic ganglia. This is likely to apply for *C. salei* as well, whose optic neuropiles are a considerable distance away from the eyes (Babu and Barth, 1984; Strausfeld and Barth, 1993).

In a few instances, a positive-going second wave was observed in the secondary eyes in their night state. No experiments were performed to trace the origin of this hyperpolarization.

The absolute values of the ERG potentials at maximum illumination intensity with white light reached 10mV, the PL eyes usually showing the highest values among all eyes. In most cases, however, the ERG amplitude measured only around 5mV. With

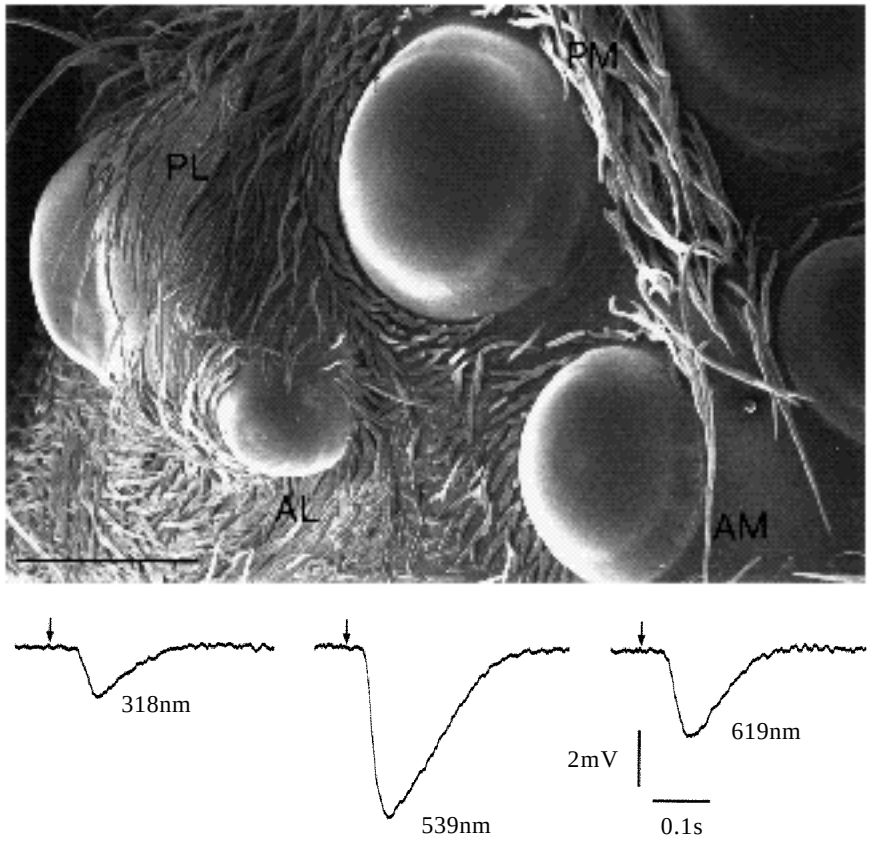

Fig. 1. Scanning electron micrograph of the eyes on the right side of an adult female *Cupiennius salei* and typical ERGs. AL, anterior lateral eye; PM, posterior median eye; PL, posterior lateral eye; AM, anterior median eye. The ERG recordings are from a PL eye and are the result of stimulation with the wavelengths indicated.

monochromatic light stimulation at the maximum intensity possible in our setup, ERGs were mostly close to 3mV.

Spectral response

A general feature of the spectral response curves of all four pairs of eyes is the presence of a green peak at 520 or 540nm and of a smaller second shoulder in the ultraviolet at approximately 380nm in the posterior eyes and at around 340nm and 360nm in the anterior eyes. The response level in the spectral range of the ultraviolet shoulder is between 65% and 80% of that of the green peak and stretches from about 340nm to 400nm (Fig. 2). According to a comparison of the spectral sensitivity curves taken during the day and at night, the spectral position of the peaks remains unchanged, whereas sensitivity increases during the dark phase. This change is bigger in the median than in the lateral eyes (Fig. 2). The relative amplitude of the ultraviolet peak increases significantly at night compared with the day in the case of AM and PM eyes. The corresponding change is not significant in AL and PL eyes, however (Table 1).

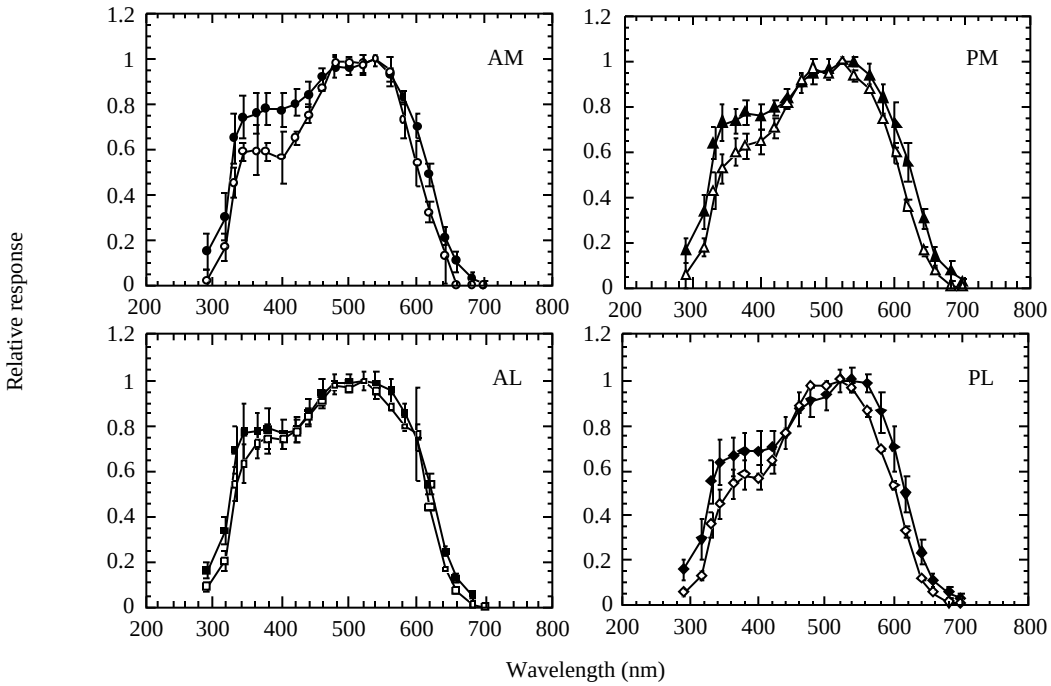

Fig. 2. Spectral response curves for all four types of eyes. Filled and open symbols refer to measurement in the subjective night and day states of the spider, respectively. Bars give S.D. (AM: ● *N*=4, *n*=12; ○ *N*=1, *n*=3) AL: ■ *N*=3, *n*=12; □ *N*=1, *n*=3; PM: ▲ *N*=4, *n*=12; △ *N*=1, *n*=3; PL: ◆ *N*=3, *n*=10; ◇ *N*=1, *n*=3).

Selective adaptation of the eyes to either ultraviolet or green light was used to get an indication of whether the spectral response curves reflect the presence of one or two, or indeed several, types of photoreceptors. To this end, the eyes were preadapted to either ultraviolet light at 364nm or to green light at 522nm for 30min before the measurement started. The intensity of the preadaptation light was set to elicit 10% of the maximum response of the respective eye achieved with white light. The preadaptation light was kept on during the measurement of the spectral response.

As shown by the examples given in Fig. 3, no evidence in favour of the presence of

Table 1. *A comparison of the relative ultraviolet responses seen in Fig. 2 at night and during the day*

	Day		Night		*P*
	n	Mean	*n*	Mean	(day *versus* night)
AM	3	0.597	12	0.747	0.026*
AL	3	0.721	12	0.757	0.484
PM	3	0.594	12	0.731	0.002*
PL	3	0.537	10	0.642	0.078

Asterisks mark significance of difference (*t*-test; $P<0.05$).
n, number of measurements.

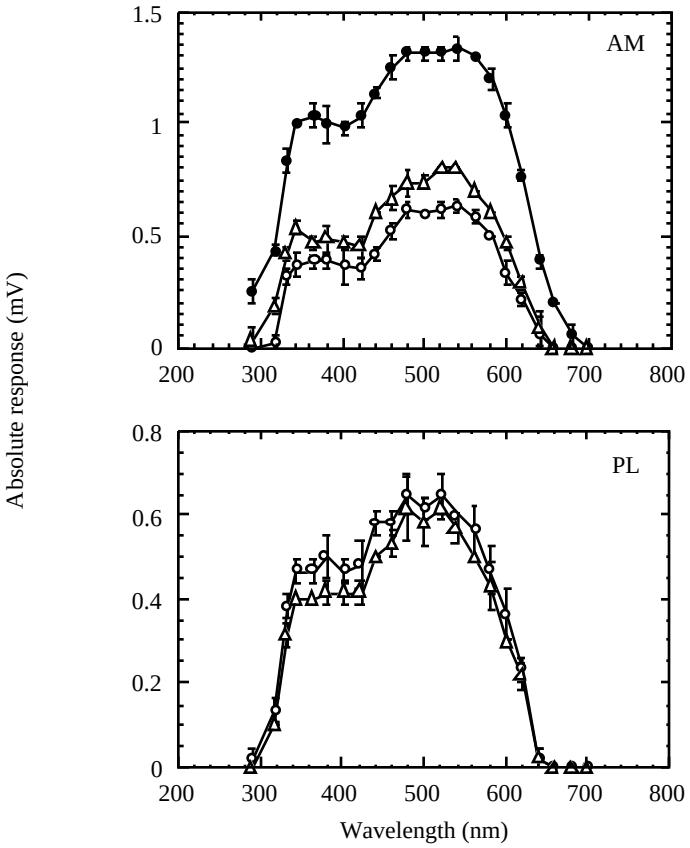

Fig. 3. Spectral response curve after selective adaptation to monochromatic light during the subjective night state of the spider (●), without adaptation to monochromatic light; ○ and △, after 30min of exposure to light of 364nm and 522nm, respectively. The intensity of the preadaptation light, which was also kept on as background illuminance during the actual measurement, corresponded to an illuminance eliciting 10% of the maximum response. All curves: values are mean ± S.D., N=1, n=3.

more than one spectral type of photoreceptor could be derived from these experiments. Absolute response values drop after selective adaptation, but the shape of the curve remains largely the same as before. There is no significant difference between the ratios of ultraviolet/green between the measurements taken after ultraviolet and green adaptation (*t*-test).

Spectral sensitivity

Fig. 4A presents a spectral sensitivity (SS) curve obtained from the conversion of a spectral response curve using the eye's V–log$I$ curves. When comparing individual values of this curve with values obtained for the corresponding wavelengths from V–log$I$ curves for monochromatic light (such as that shown in Fig. 6), a very good coincidence is seen (Fig. 4A). This finding points to identical slopes of all the V–log$I$ curves for

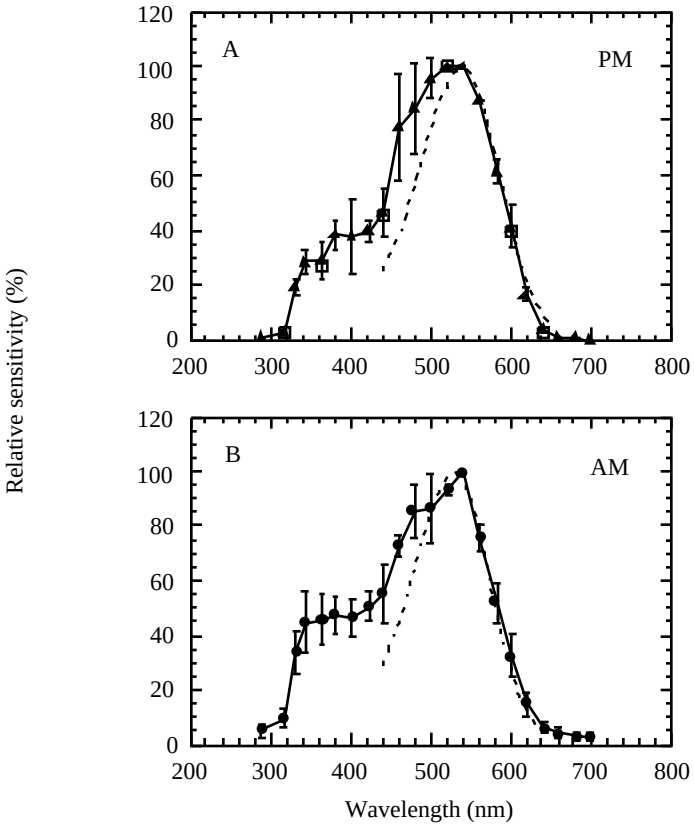

Fig. 4. (A) Spectral sensitivity (SS) curve of a PM eye (night state) obtained from spectral response curves for monochromatic light such as those presented in Fig. 6. Squares show values calculated using the slope of monochromatic *V*–log*I* curves. (B) SS curve for AM eyes (night state) (*N*=3, *n*=9; S.D. given). The dashed lines give the predicted absorption spectrum from a Dartnall nomogram with the peak absorption wavelength at 540nm (A) and 530nm (B).

monochromatic light and for white light. Consequently, *V*–log*I* curves for any wavelength or for white light can be used for the conversion of spectral response curves into SS curves.

Dartnall nomograms plotted onto SS curves (Fig. 4) fit the SS curves quite well at wavelengths longer than 530nm in all four pairs of eyes. We conclude that a single visual pigment is responsible for the response in the spectral range between 530nm and 650nm. In the range from 450nm to 500nm, the Dartnall curve clearly deviates from the SS curve. The SS curves of all eyes have a small shoulder at 480nm, which indicates the possible existence of a visual pigment maximally absorbing in the blue. Since there is also a peak in the ultraviolet, trichromatic colour vision is a possibility. However, sensitivity in the ultraviolet may also be due to the secondary peaks of green receptors, as described in insect compound eyes such as those of dragonfly (Eguchi, 1971), locust (Bennett *et al.* 1964), fruitfly (Kirschfeld *et al.* 1977) and butterfly (Bandai *et al.* 1992).

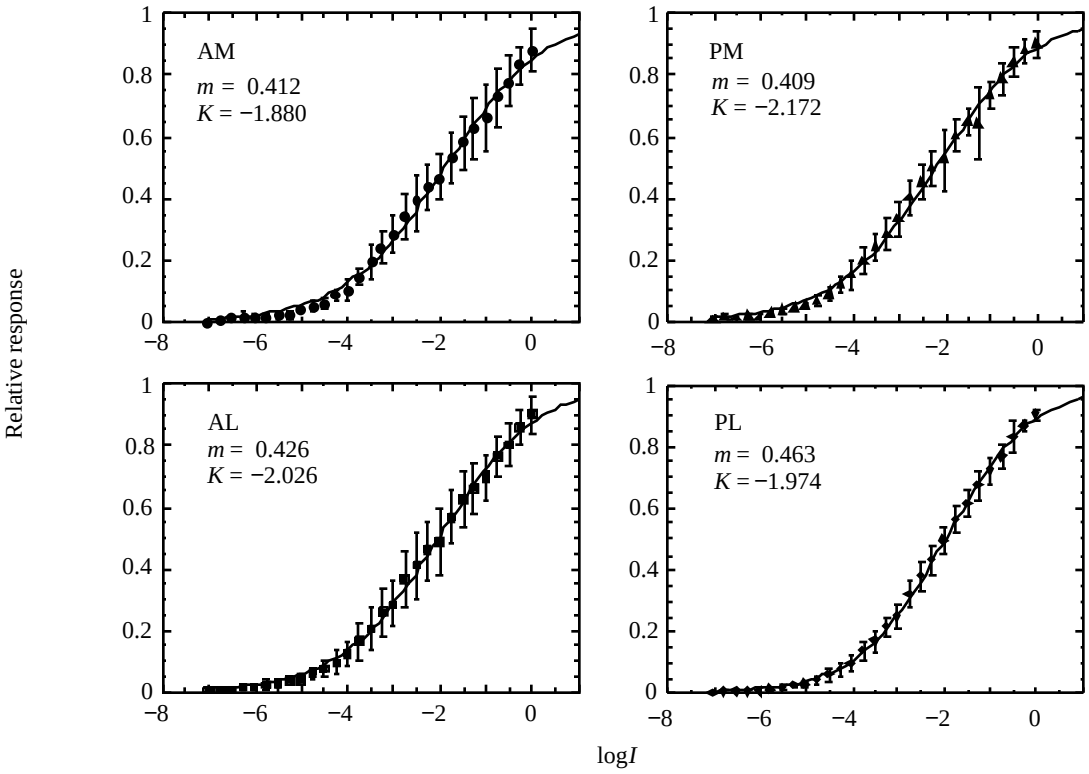

Fig. 5. Intensity curves measured with white light for AM, PM, AL and PL eyes, all in their night state. The highest relative (ordinate) response was set to 1 and is estimated from the theoretical Naka–Rushton equation (see text). The insets give the slope of the curve (*m*) and the light intensity at $V_{max}/2$ (*K*). For all eyes, values are mean ± S.D., *N*=2 and *n*=6.

Intensity curves and absolute sensitivity

White light

In all eyes, an ERG response is seen at stimulus intensities as small as 6–7logunits down from the maximum intensity possible in the experiments (log*I*=0), which was 900lx. With the luxmeter available, we could measure illuminance values down to −4.75log*I*, where they amounted to 0.01lx. Thresholds were clearly below this value.

The log–linear response range of the eyes covers a stimulus intensity range of 4 logunits. When plotting the original absolute response values, also used for Fig. 5, it can be seen that the slope of the intensity curve is steepest in the PL eye and flattest in the AM eye. This difference is statistically significant (*t*-test; *P*=0.017). It implies a smaller dynamic range in the PL than in the AM eyes. The PM and the AL eyes show values for the slope which are intermediate between those of the AM and PL eyes. According to statistical tests (Table 2), the PL eyes differ significantly from all other eyes regarding slope (*m*), whereas *m* does not differ for AM, AL and PM eyes. The statistical tests also show that the values for $V_{max}/2$ (*K*), referred to as relative sensitivity, do not differ significantly among any of the eyes.

Table 2. *Comparison and statistical significance of the slopes (*m*) and of the* $V_{max}/2$ *values (*K*) of the response curves shown in Fig. 5*

	Number of experiments	Mean of slope (*m*)	*P*			Mean of sensitivity (*K*)	*P*		
			AL	PM	PL		AL	PM	PL
AM	6	0.412	0.392	0.830	0.011*	−1.880	0.543	0.167	0.608
AL	6	0.426		0.111	0.006*	−2.026		0.482	0.781
PM	6	0.409			0.001*	−2.172			0.171
PL	6	0.463				−1.974			

Asterisks mark significance of difference (*t*-test; $P<0.05$).

Monochromatic light

Stimulation with monochromatic light at six different wavelengths was used to determine whether there are obvious differences in slope of the intensity curves. As shown by the representative example in Fig. 6, there are no such differences. This implies that, despite differences in threshold and relative sensitivity, the spectral sensitivity function $S(\lambda)$ can be expected to remain the same for different response levels.

Naka–Rushton equation and sensitivity changes

The V–log$I$ curve of the ERGs of the eyes of *Cupiennius* follows the equation given by Naka and Rushton (1966*a*,*b*) very well:

$$V_I = I^m \times V_{max}/(I^m + K^m).$$

In this equation I is the stimulus intensity, V_I is the response amplitude at intensity I, m represents the slope of the linear part of the V–log$I$ curve and K the stimulus intensity eliciting half of the maximum response (V_{max}).

Cupiennius salei is a nocturnal spider whose locomotor activity starts within less than

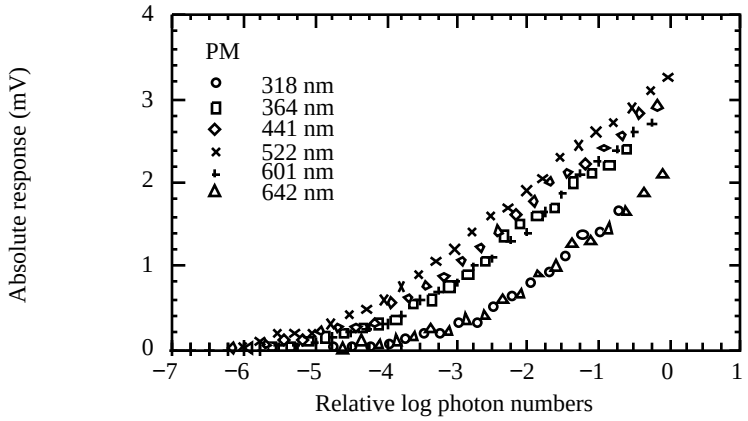

Fig. 6. Intensity curves for a PM eye in its night state when stimulated with different wavelengths.

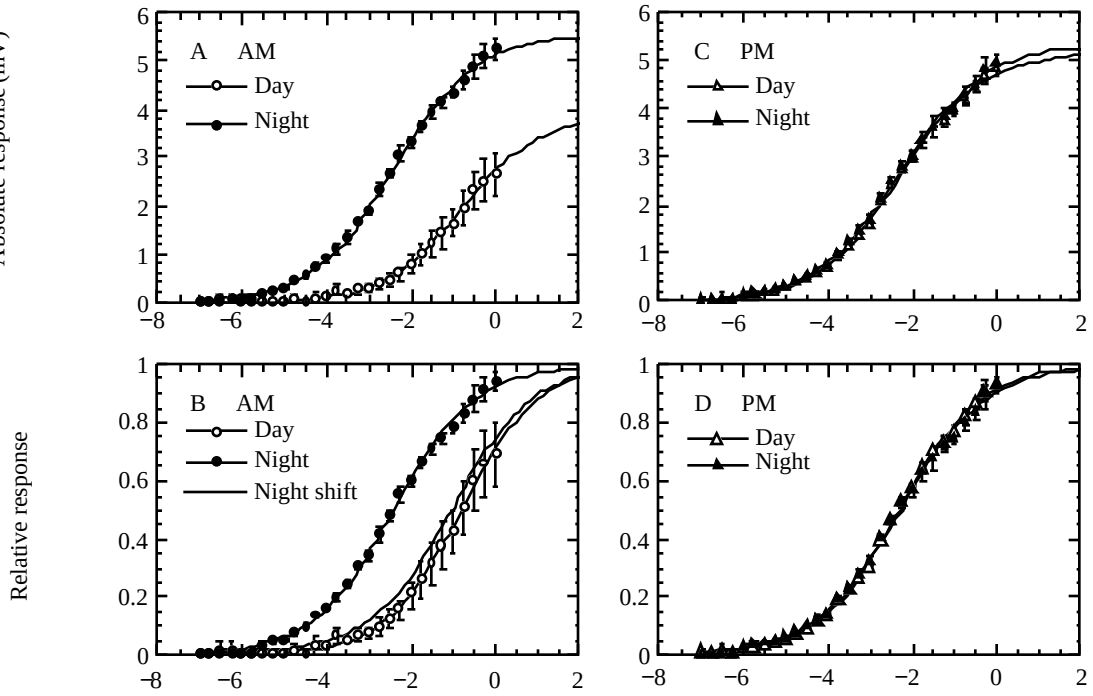

Fig. 7. (A–D) Intensity curves for an AM (A,B) and a PM (C,D) eye measured during the day and at night. Note the shift in sensitivity in the case of the AM eye and its absence in the PM eye. The night-time *V*–log*I* curve calculated according to the Naka–Rushton equation (thick line, see text) was shifted horizontally in B to the point where the value 0.95 of the relative response at night coincided with the value 0.95 of the relative response of the daytime curve. Values are mean ± S.D., N=1, n=3.

an hour after sundown (Barth and Seyfarth, 1979; Seyfarth, 1980; Schmitt *et al.* 1990). We find that the responses of the eyes change with the time of day. Sensitivity in the AM eyes increases at night compared with daytime. Taking K as a measure of sensitivity, the night-time eye is up to 0.81logunits (1.97–1.16) more sensitive than the daytime eye. Such an increase in sensitivity may be brought about by a decrease in m (which is typical of invertebrates), by a shift of the curve towards lower intensity values (which is typical of vertebrates) or, indeed, by both of these mechanisms (Eguchi and Horikoshi, 1984). The average value of m representing the slope for nine AM eyes was 0.47 for the day and 0.42 for the night. However, in addition to a decrease in the slope, the curve also shifts towards lower values at night. In other cases of AM eyes, the shift was more prominent and indeed the main reason for the observed increase in sensitivity. Fig. 7A,B shows *V*–log*I* curves for an AM eye and gives the relative and the absolute response values for both the day and the night. In addition, the corresponding theoretical curves derived from the Naka–Rushton equation are presented. It can be clearly seen that the increase in sensitivity at night in this case is mainly due to a horizontal shift of the daytime curve towards lower stimulus intensity values but not to a decrease in the slopes.

Contrasting with the AM eyes, some of the PM eyes examined showed the same sensitivity (light intensity at $V_{max}/2$) at night and during the day. The average slopes (N=6) of the intensity curves were only slightly smaller at night (0.43) than during the day (0.45) (Fig. 7C,D).

Absolute values of ERG

The absolute responses of the AM eyes recorded at night are about twice as large as those recorded during the day (Fig. 7A). Threshold illumination shifts by about 1logunit towards lower values.

Photopigment

Using HPLC techniques, the chromophore of the visual pigment of all the eyes of *Cupiennius salei* could be identified as 11-*cis* retinal (Fig. 8A,B). According to the results shown in Fig. 8C,D, there is no 3-hydroxyretinal in any of the eyes of this spider species. In addition, no dehydroretinal was detected, using crayfish (*Procambarus clarki*) chromophore as a standard. The presence of nearly the same amounts of 11-*cis* and all-*trans* isomers as well as the presence of 13-*cis* retinal (as seen in Fig. 8A) is noted for light-adapted eyes. A high peak of 11-*cis* retinol is also typical of these eyes.

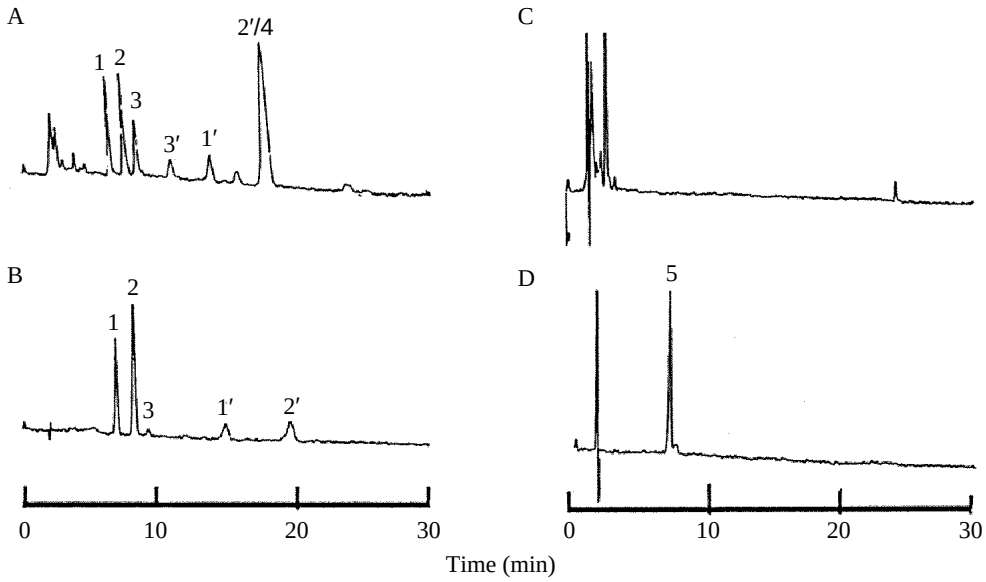

Fig. 8. (A–D) Chromatograms taken from all eight eyes of *Cupiennius salei* (A,C) and from the corresponding standards for retinal (B, commercially available) and for hydroxyretinal (D, butterfly *Papilio*, Seki *et al.* 1987). The numbers given to the peaks on the chromatograms indicate the following substances: 1, 11-*cis* retinaloxime (*syn* isomer); 2, all-*trans* retinaloxime (*syn* isomer); 3, 13-*cis* retinaloxime (*syn* isomer); 4, 11-*cis* retinol or 13-*cis* retinol; 5, all-*trans* 3-hydroxy retinaloxime (*syn* isomer); 1′, 11-*cis* retinaloxime (*anti* isomer); 2′, all-*trans* retinaloxime (*anti* isomer); 3′, 13-*cis* retinaloxime (*anti* isomer).

Discussion

Spectral sensitivity

There are both ERG studies and intracellular recordings of the spectral sensitivity of spider eyes. The data so far available are from representatives of four spider families vastly differing both in life style and with regard to the behavioural significance of their vision: orb weavers (Argiopidae), net-casting spiders (Dinopidae), hunting spiders (Lycosidae) and jumping spiders (Salticidae) (see review by Yamashita, 1985). As one would expect, spectral sensitivities differ with regard to both the taxonomic affiliation and the type of eye (principal or secondary) considered. *Cupiennius* is a genus closely related to the wolf spiders (Lycosidae), both taxonomically (Homann, 1961, 1966, 1971; Lachmuth *et al.* 1985) and behaviourally. The various species are hunting or wandering spiders. In the principal eyes (AM) of wolf spiders (*Lycosa baltimoriana, L. lenta, L. miami*), only one type of receptor cell was found (De Voe, 1972). It responded maximally at 510nm (green) and at 360–370nm (ultraviolet). Intracellular recordings from photoreceptor cells of a secondary eye (AL) demonstrated the presence of only one type of cell, with the main response maximum at 510nm and a secondary maximum at 380nm (De Voe, 1972). This is in good agreement with earlier ERG recordings in the same species from all three secondary eyes (AL, PM, PL) and with the absence of selective adaptation effects (De Voe *et al.* 1969). In summary, the data presently available point to the existence of only one population of receptor cells in all of the lycosid eyes and, consequently, to the absence of colour vision.

There are obvious similarities between these findings in lycosids and our data on the ctenid *Cupiennius salei.* (i) One similarity is the spectral range (approximately 300–680 nm) eliciting a response. The behavioural significance of the sensitivity of the eyes of *C. salei* in the ultraviolet is not clear. Photographs of male and female *C. salei* and *C. coccineus* made with ultraviolet light did not disclose any ultraviolet reflection patterns. Light coming directly from the moon contains a relatively large amount of short wavelengths below 450nm (Menzel, 1979). It is not known, however, whether *C. salei* makes use of those wavelengths behaviourally, e.g. for orientation. Sensitivity in the green, in contrast, is more easily understood since light reflected from leaves and the soil is dominated by wavelengths longer than 450nm, i.e. green and yellow (Menzel, 1979). (ii) Another similarity to the lycosids is the dominance of the green peak (520 or 540 nm) in the spectral response curve and the existence of a shoulder in the ultraviolet (PM, PL: approximately 380nm; AM, AL: approximately 360nm and 340nm). We never found a dominant maximum in the ultraviolet, however, as was the case in the lycosid AM eyes. Also, the green peak is slightly shifted towards longer wavelengths compared with the peak in the lycosids. (iii) The position of the maximum relative sensitivities at the above-mentioned wavelengths remained unchanged by chromatic adaptation.

The conclusion from these observations is that there is no compelling evidence from our ERG study in favour of colour vision in *C. salei*, either in the principal or the secondary eyes. We note, however, that the deviation of the Dartnall curve from the SS curves between 450nm and 500nm and a small but consistent shoulder on the SS curves

in the blue at 480nm leave the possibility of the existence of more than one spectral type of photoreceptor cell.

Considering the predominantly nocturnal activity of *Cupiennius salei* (Barth and Seyfarth, 1979; Schmitt *et al.* 1990), a lack of colour vision would not be surprising. So far, however, too little is known about the behavioural role of vision in *Cupiennius salei*, which has well-developed eyes (Land and Barth, 1992) and optic ganglia (Strausfeld and Barth, 1993; Strausfeld *et al.* 1993). In *Dinopis subrufus*, the enormously enlarged PM eyes, which are specialized for extreme sensitivity and used to capture prey at night at very low luminance levels, have only green receptors (Laughlin *et al.* 1980). The same is true for all other secondary eyes so far studied in any spider and has been taken to indicate colour blindness. The only known exceptions are the PL eyes of several species of orb-weaving *Argiope*. They have ultraviolet, green and blue receptors, not unlike the principal eyes (AM) of the same species and the principal eyes of salticids, which have di-, tri- or tetrachromatic vision and are thought to be capable of colour vision (see review by Yamashita, 1985).

Absolute sensitivity and V–*log*I *functions*

Our present ERG data strongly support the possibility that *Cupiennius salei* does indeed use its visual capabilities, despite being a largely nocturnal species and therefore most active at times of very poor illumination conditions. Our results also point to differences in functional specialization among the four pairs of eyes. Judging from the *V*–log*I* curves (Fig. 5) and from the sensitivity values *K* (representing $V_{max}/2$) (Table 2), all eyes are about equally sensitive at night. There are some differences between the eyes, however. The slope of the *V*–log*I* curve is steepest in the PL eyes. The AM eyes, in contrast, seem to be the only ones capable of adjusting to daytime light conditions by shifting their *V*–log*I* curve towards higher values of light intensity (Fig. 7). In this regard it should be remembered that the AM eyes are the only ones not having a reflecting tapetum and that it was for them that the lowest *K* value (Table 2) was found.

According to field observations of *Cupiennius salei* in its natural habitat in Guatemala (Barth and Seyfarth, 1979; Seyfarth, 1980), the spiders do not leave their retreats until nightfall. After sunset, at illumination intensities of about 15lx, *Cupiennius salei* turns around abruptly above its retreat so that its prosoma points upwards. It then remains motionless for about half an hour until darkness is complete (for the human eye) and starts moving slowly up a leaf to wait for prey at illumination levels below 0.1lx. The ERG threshold intensities found in this study using white light stimuli were below 0.01lx, i.e. well within these ranges. Similar field observations are now available for *C. coccineus* in Costa Rica, a close relative of *Cupiennius salei* with very similar behaviour and habitat (F. G. Barth, D. Baurecht and A. Schmitt, in preparation). According to a recent study of the optical performance of the eyes of *Cupiennius salei* (Land and Barth, 1992), F-numbers of the lenses (*f*/*D*; *f*, focal length; *D*, lens diameter) are all between 0.58 and 0.74, i.e. impressively low and providing bright images. In addition, all secondary eyes have a gridiron tapetum. The tapetum doubles the effective length of the rhabdoms, which receive the light both directly and after reflection from the tapetum. Both these circumstances are thought to contribute to the sensitivity of the eyes of *Cupiennius salei*.

A behavioural role of vision must be taken into account not only shortly after sundown, however. A corneal illuminance of 3×10^{12} photons$\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$ at half-maximal response to monochromatic light (522nm) and one of 3×10^{9} photons$\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$ at threshold indicates a sensitivity high enough to permit vision under conditions of moonlight and clear sky. The illuminance of the corneal surface of a spider eye that is irradiated by moonlight reflected from a white card (diameter 3mm and assumed to correspond to the tip diameter of the light guide used in our experiments) can be calculated from the luminance data and equations given by Land (1981) as 7.85×10^{9} photons$\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$. The equivalent value for reflected starlight is 7.85×10^{7} photons$\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$. Thus, under optimal conditions, the threshold intensity found for *Cupiennius* eyes suggests that vision is possible in moonlight but not in starlight. The fact that the values compared refer to 555nm (moonlight) and 522nm (λ_{max}, the most effective wavelength of the spider eye), respectively, does not affect the argument, since the corresponding change in sensitivity amounts to only about 5%. In the end, however, it is the *retinal* illuminance that determines the visual response. According to Land (1981), retinal illuminance $J_R=J_S(D^2\times A_p)/(A_1\times f^2)$, (where J_S is surface illuminance, A_p is the area of the pupil, D is the diameter of the lens, A_1 is the area of the tip of the light guide and f is the distance between pupil and retina. Values for A_p and f are taken from Land and Barth, 1992). *Retinal* illuminance at threshold can be calculated to be 5.9×10^{9} photons$\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$, i.e. roughly twice as high as the illumination at the corneal surface. This does not change our argument. Absolute sensitivity may turn out to be even higher when measured with more sensitive methods, such as single-cell recording. The values now available are about 10^7 times higher than those given for the PM eyes of *Dinopis* (Laughlin *et al.* 1980) and 10^2 times higher than those given for the PL eyes of the diurnal jumping spider *Plexippus* (Hardie and Duelli, 1978). Thus, the nocturnal net-casting spider *Dinopis* clearly remains the champion.

Photopigment

In our search for chromophores of the photopigments in the eight eyes of *Cupiennius salei* we found only retinal; there was no dehydroretinal or hydroxyretinal. Accordingly, retinal is taken to be the chromophore in both the principal and the secondary eyes. 11-*cis* retinal was also reported for another spider, *Cryophoeca montana*, by Smith and Goldsmith (1990). As shown in the chromatogram of Fig. 8A, a peak of 11-*cis* retinol is prominent and higher than those of 11-*cis* retinal and all-*trans* retinal. A large amount of 11-*cis* retinol in the retina probably indicates the storage of a precursor of 11-*cis* retinal. It may also suggest that retinol is a sensitizing pigment responsible for the ultraviolet sensitivity (secondary peak) like that found for a green receptor in the fly eye (Kirschfeld *et al.* 1977; Vogt and Kirschfeld, 1983).

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