J Comp Physiol A (1995) 176: 437–453

ORIGINAL PAPER

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The dorsal eye of the dragonfly *Sympetrum*: specializations for prey detection against the blue sky

Accepted: 26 August 1994

Abstract Dragonflies of the genus *Sympetrum* have compound eyes conspicuously divided into dorsal and ventral regions. Using anatomical, optical, electrophysio-logical, in-vivo photochemical and microspectrophotometrical methods, we have investigated the design and physiology of the dorsal part which is characterized by a pale yellow-orange screening pigment and extremely large facets . The upper part of the yellow dorsal region is a pronounced fovea with interommatidial angles approaching 0.3°, contrasting to the much larger values of 1.5°–2° in the rest of the eye. The dorsal eye part is exclusively sensitive to short wavelengths (below 520 nm). It contains predominantly blue-receptors with a sensitivity maximum at 420 nm, and a smaller amount of UV-receptors. The metarhodopsin of the blue-receptors absorbs maximally at 535 nm. The yellow screening pigment transmits long-wavelength light (cut-on 580 nm), which increases the conversion rate from metarhodopsin to rhodopsin (see Fig. 11a). We demonstrate that because of the yellow pigment screen nearly all of the photopigment is in the rhodopsin state under natural conditions, thus maximizing sensitivity. Theoretical considerations show that the extremely long rhabdoms (1.1 mm) in the dorsal fovea are motivated for absorption reasons alone. A surprising consequence of the long rhabdoms is that the sensitivity gain, caused by pumping photopigment into the rhodopsin state, is small. To explain this puzzling fact we present arguments for a mechanism producing a gradient of rhodopsin concentration along the rhabdom, which would minimize saturation of transduction units, and hence improve the signal-to-noise ratio at high intensities. The latter is of special importance for the short integration time and high contrast sensitivity these animals need for spotting small prey at long distances.

Key words Compound eye · Dragonfly · Electrophysiology · Optics · Photochemistry

Abbreviations *ERG* electroretinogram · *R* rhodopsin · *M* metarhodopsin

Introduction

For most flying animals the horizon divides the visual world into quite different halves. The ground provides the image texture necessary for detecting self motion, it contains a wealth of navigational cues, and colour information is often abundant. The visual information content in the sky is entirely different and much more sparse. Apart from solar position and sky polarization, which enables visual compass orientation in insects, the most obvious visual cues in the sky are other flying animals. A large number of insects detect mates or prey against the sky, and it is not uncommon for these animals to have specialized dorsal eye-regions. The most prominent examples are found in mayflies, dragonflies, owlflies, simuliid and bibionid flies, and drone honey-bees (reviewed by Wehner 1981; Land 1989).

Detection of a flying insect requires high spatial resolution. An object, the size of a housefly (6 mm), subtends an angle of less than 0.35° at the distance of 1 m. This is within the immediate flying range of many large insects, but the resolution required to see the object is only just possible with a compound eye. For detection of fast flying objects it is also necessary that the visual system has a short integration time, and this can be realized only if the eye's sensitivity allows

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a sufficient photon catch. High resolution and high sensitivity in a compound eye requires numerous and large facets, which inevitably makes the eye large. Indeed, large dorsal eyes are characteristic for those insects that rely on vision for detecting prey or mates against the sky (Wehner 1981; Land 1989).

Another conspicuous characteristic of these dorsal eye regions is their pale, often red or yellow pigmentation, which contrasts with the much darker ventral part of the eye (e.g. simuliid flies: Kirschfeld and Wenk 1976; mayflies: Horridge 1976; Horridge and McLean 1978; Horridge et al. 1982; drone honey-bee: Menzel et al. 1991; reviews by Wehner 1981, and Stavenga 1992). The spectral sensitivity is often different between the dorsal and ventral regions: a typical dorsal eye has no sensitivity outside the UV-blue range, whereas the sensitivity of the ventral eye may extend through the entire spectrum up to yellow or sometimes even red (e.g. drone honey-bee: Bertrand et al. 1979; Menzel et al. 1991; owlflies: Gogala 1967; Gogala et al. 1970; libellulid dragonflies: Mazokin-Porshniakov 1959; Ruck 1965; Horridge 1969; Meinertzhagen et al. 1983; review by Stavenga 1992). The presence of exclusively short wavelength sensitivity in the dorsal part of the eye is not surprising since this part of the eye is viewing the sky, which is dominated by short wavelengths.

Insects, like other invertebrates, have bistable photopigments: when a photopigment molecule (rhodopsin) absorbs a photon, it converts into a thermostable metarhodopsin, which in turn can be reconverted into the original rhodopsin by the absorption of yet another photon. Rhodopsins sensitive to short wavelengths generally produce metarhodopsins with absorbance maxima at longer wavelengths (Stavenga 1989). For instance, in the dorsal eye of the drone honey-bee a rhodopsin absorbing maximally at 446 nm has a metarhodopsin peaking at 505 nm (Bertrand et al. 1979; Menzel et al. 1991), and in the owlfly *Ascalaphus* the corresponding shift is from 345 nm to 475 nm (Gogala et al. 1970; Hamdorf et al. 1973). The combination of short-wavelength sensitivity and photopigment regeneration with long wavelengths is the probable reason for the red, orange or yellow screening pigment in these dorsal eyes (originally proposed by Schneider et al. 1978 and Stavenga 1979; for recent review see Stavenga 1992). For long wavelength light the yellow screening pigment is translucent, which greatly increases the amount of light available for photopigment regeneration. This does not compromise resolution, however, because for blue and ultraviolet light the screening pigment remains dark, and thus acts the same way as the brown screening pigment does in the ventral part of the eye.

The fact that the optics is different for long and short wavelengths raises an obvious question: to what extent does this arrangement improve the performance of the eye? Dragonflies of the genus *Sympetrum* offer ideal experimental animals for addressing this question. They are long-range predators, which use their extremely large compound eyes to locate flying prey against the sky. The dorsal part of their eyes is differently coloured compared to the ventral part, indicating the presence of a yellow screening pigment. In this paper we have used optical and electrophysiological methods to investigate the optics of the *Sympetrum* eye at both short and long wavelengths, and to study the photochemistry of the eye in vivo.

Material and methods

Animals

Dragonflies of two species were used for this investigation: *Sympetrum striolatum* and *Sympetrum vulgatum* (Libellulidae). The animals were caught wild in the vicinity of Zürich, Switzerland (for histology and electrophysiology) and Lund, Sweden (for morphology, optics and microspectrophotometry). Animals not used immediately were stored at 14°C under normal light/dark conditions, and regularly fed with fruitflies.

Histology

Histological procedures were as described by Meyer and Labhart (1993). Investigations of the distribution of screening pigment were made by light microscopy of unstained 2 μm sections. Rhabdom shapes were determined from stained cross-sections .

Optics

Optical experiments were carried out on an ophthalmoscopic setup described in detail elsewhere (Nilsson and Howard 1989). Only a brief description will be given here. The setup was built around a Zeiss photomicroscope, equipped with an epi-illumination attachment modified such that light could enter the microscope via an external optical bench. The illumination beam path on the optical bench had three alternative light sources: a 50 W halogen lamp, a 75 W Xenon arc lamp, and a 1 mW HeNe laser (633 nm). Illumination intensity was adjusted with a neutral density wedge, and spectral filtering was performed by a set of Schott narrow-band interference filters, or a set of short-pass interference filters (Corion) and long-pass coloured glass filters (Schott). Adjustable diaphragms were arranged such that one was imaged at the microscope focal plane, and another at infinity, providing full control of both the area and angle of illumination. A half silvered mirror close to the back of the microscope objective linked the illumination beam path to the observation beam path of the microscope, thus producing incident illumination. For some experiments the illumination beam path was altered to enter below the specimen as in a conventional compound microscope. The images produced in the microscope could either be viewed and photographed directly, or first intensified through a Hamamatsu image intensifier (V 1366 P). It was also possible to direct the image to a cooled photomultiplier tube (Hamamatsu R 2949 HA) for intensity measurements.

Electrophysiology

Electrophysiological recordings were made on dragonflies mounted with wax, with the eye under study centered in a perimeter holding

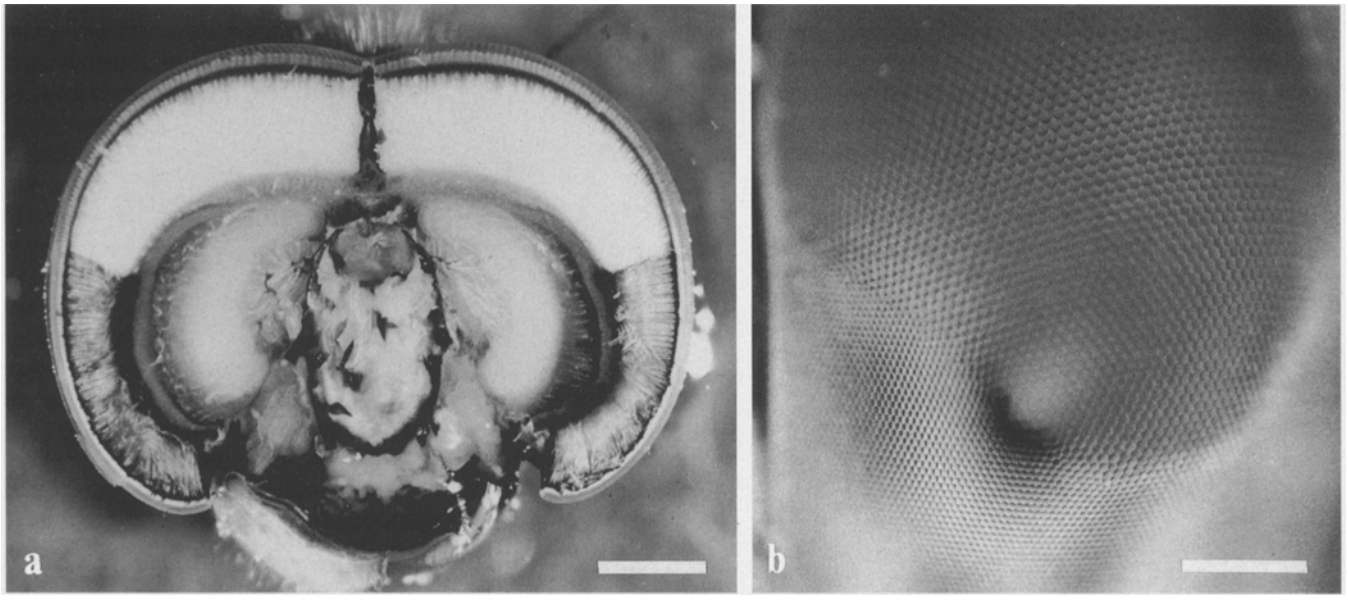

Fig. 1a, b The dorsal-ventral division of the compound eye of *Sympetrum*. **a** Hemisected head of a fresh dragonfly, illustrating the sharp division in pigmentation between ventral and dorsal. The screening pigment in the dorsal region is yellow-orange, whereas it is black in the ventral part (partly masked by reflecting tracheoles). **b** The pseudopupil at the border between the dorsal and ventral regions, observed with incident light. In the dorsal part the pseudopupil is glowing orange on a darker background, but in the ventral part it is black on a pale grey background. *Scale bars*: **a** 1 mm; **b** 0.5 mm

the illuminating light-guides. For *intracellular recordings* 2 M KCl-filled micropipettes were introduced in the eye through a small opening cut into the cornea as far away from the eye region of interest as possible. Receptor potentials were recorded with a high impedance electrometer (M-707, WPI). For recordings of *electroretinograms* (ERGs), a mechanically sharpened steel electrode was inserted in the most ventral section of the yellow-pigmented dorsal half of the eye. The compound eyes and the ocelli were occluded with black paint, except for a circular area in the centre of the dorsal fovea of the recorded eye (12–18 ommatidia in diameter), and a small spot for the ERG recording electrode, which was shielded from light. ERGs were recorded with a low-noise operational amplifier (LF 441, National Semiconductor Corp.) in AC mode with a 100 Hz low pass filter. Both for intracellular recordings and for ERGs the signals were displayed on an oscilloscope and recorded on a high frequency chart recorder (Mark 220, Gould). Response amplitudes were measured either from the hard copies or on-line with a peak detector.

Light was supplied by a 900 W Xenon arc lamp feeding into two beam paths: one for stimulation and one for adaptation. The *stimulation* path was equipped with a set of 16 narrow-band interference filters for generating monochromatic light (adjusted to equal quanta $\pm$ 0.1 log unit) and a Schott glass filter (BG 12) for wide-band blue stimuli. The intensity was controlled by a quartz neutral density wedge. The light was focused into a flexible UV-transmitting light-guide, the other end of which was mounted on the arm of the perimeter. The beam path for *adaptation* light provided wide band blue or yellow light (BG 12 or OG 550 Schott filters, respectively). The intensity was controlled by a set of 6 quartz neutral density filters. For most experiments this light path was combined with the stimulus path via a half-mirror, such that the same light guide delivered both stimulation and adaptation light. For experiments where the yellow adaptation light was given at varying angular positions (for measuring angular sensitivity of recovery), it entered a separate light-guide which ended on a small accessory perimeter, mounted on the main perimeter arm. Both light paths were equipped with quartz optics and electromagnetic shutters. The angular size of the test and adaptation flashes ranged between 0.2° and 2.9°; small sizes were used for measuring spatial properties of the eye (relevant details are given in the Results section). The duration of the test flashes was 100 ms, except for some ERG experiments where it was 60 ms. Except for measuring angular sensitivities, the experiments were performed with the stimulus centered in the visual field of a receptor (intracellular recordings) or of the exposed spot of fovea (ERG) as defined by maximal response amplitude. In the in vivo photochemistry experiments a continuous train of test flashes (intervals 5 or 6 s) served to probe sensitivity changes induced by actinic irradiation (for further details see results). Spectral and angular sensitivities were calculated from the corresponding efficiency measurements and the intensity characteristic of the electrical reponse (for visual sensitivity) or the intensity characteristic of response recovery (for sensitivity of $M \rightarrow R$ conversion), which were measured along with the efficiency measurements. Spectral sensitivities were corrected for the exact intensity value of each spectral stimulus. In angular sensitivity measurements, in which the stimulus did not move along a great circle, the perimeter readings were appropriately corrected (Burkhardt and Streck 1965).

Results

Anatomical specializations

The eyes of the two *Sympetrum* species *striolatum* and *vulgatum* are almost identical. There is a conspicuous division into a ventral half with small facets and a dorsal half with much larger facets. A vertical cut through a fresh eye (Fig. 1a) shows that the two halves differ markedly also in pigmentation. The ventral part contains dark pigment, as in most other apposition eyes. But the dorsal part contains screening pigment of a light yellow-orange colour, large granules of which are concentrated around the crystalline cones and near

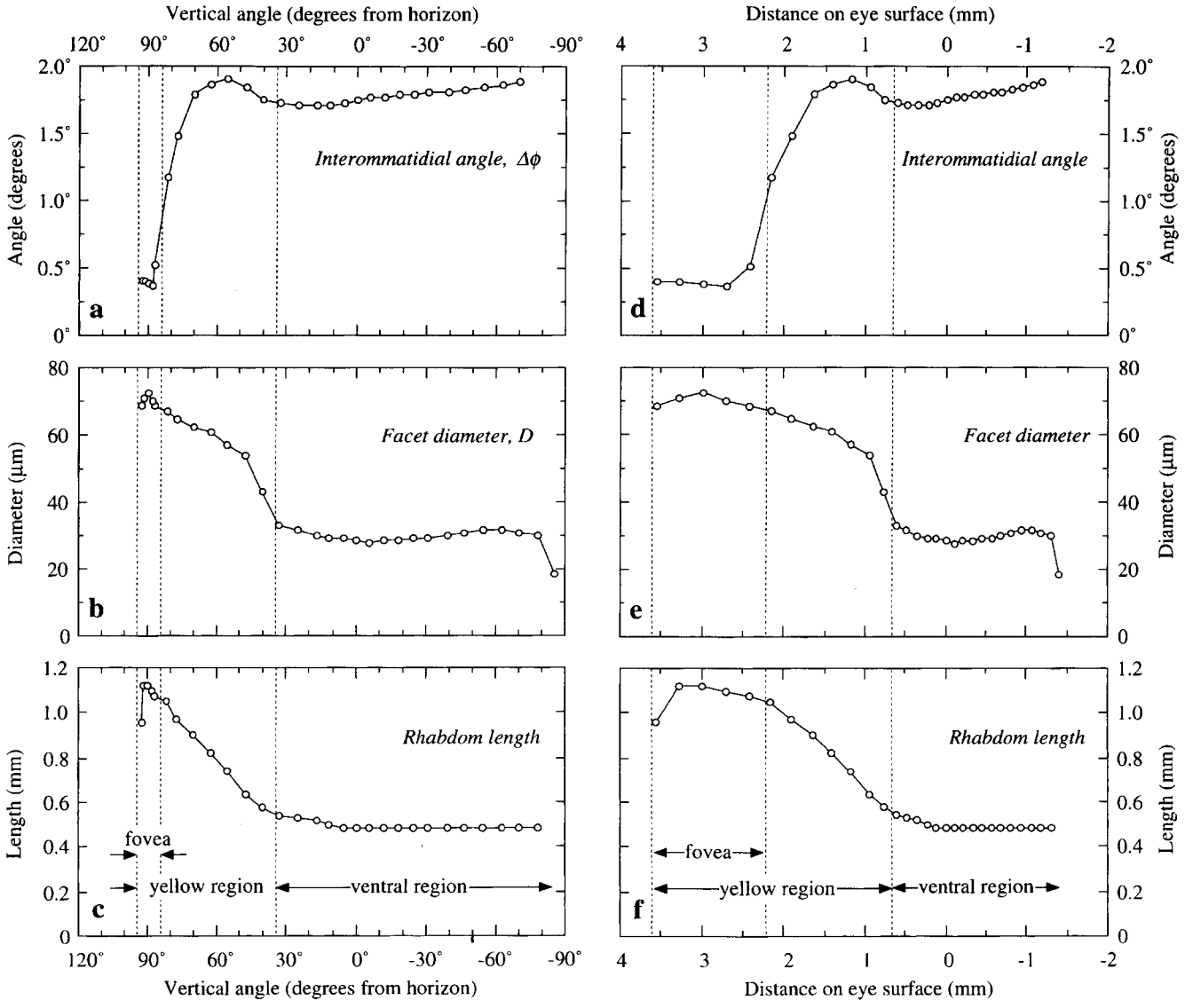

Fig. 2a–f The variation of optically important parameters of the eye along the vertical axis. Measurements were made along a line from the dorsal to the ventral edge of the eye, intermediate between frontal and lateral. The interommatidial angle is plotted in **a** and **d**, the facet diameter in **b** and **e**, and the rhabdom length in **c** and **f**. All three parameters show variations which divide the eye into a homogeneous ventral region and a yellow dorsal region, part of which is a pronounced dorsal fovea. The large facets and small interommatidial angles of the fovea makes it appear small when plotted against vertical angle (**a**–**c**), but large when plotted against distance on the eye surface (**d**–**f**)

the basement membrane. Smaller sized yellow pigment granules are present in the receptor cells all along their length, but thinning out from high concentration below the crystalline cone towards deeper layers of the retina. In the hemisected eye (Fig. 1a), the bulk of the dorsal retina appears pale yellow with a marked distal and a thin proximal orange layer. The pigmentation division between the ventral and dorsal parts is very sharp and coincides exactly with the change in facet diameter.

The differences between dorsal and ventral are clearly visible also by the external colouration of the live eye. Using incident light, the ventral part displays a black pseudopupil surrounded by dimmer accessory pseudopupils, all on a gray background, whereas in the dorsal part the contrast is reversed, with the pseudopupil glowing pale orange on a darker orange-brown background (Fig. 1b) (see Stavenga 1979). The pseudopupil seen from straight above the animal is more than four times larger than anywhere else in the eye, indicating the presence of a pronounced dorsal fovea.

To quantify the regional variations in the eye, a dragonfly was mounted in a goniometer, and photographed through a microscope as the goniometer was turned in 5° intervals. The goniometer was turned such that the pseudopupil followed a line from the dorsal to the ventral edge of the eye, intermediate between frontal

and lateral. The photographs were used to measure interommatidial angle and facet diameter as a function of vertical angle in the visual field. The results show that the interommatidial angle stays rather constant between 1.5° and 2° in most of the eye, but drops to less than 0.5° in a well-defined fovea pointing straight up (Fig. 2a). The facet diameter is surprisingly uniform in the ventral part of the eye (about 30 μm) but increases rapidly in the yellow region, to reach a peak value of more than 70 μm in the ommatidia pointing 90° up (Fig. 2b). These measurements were compared to the rhabdom length, measured on a vertical section through the eye. The rhabdoms are unusually long in the entire eye with the most extreme values of more than 1 mm in the dorsal fovea (Fig. 2c). These measurements indicate the presence of three functionally distinct regions in the eye: (1) a normally pigmented ventral area with relatively large interommatidial angles and small facets; (2) a yellow dorsal region with relatively large interommatidial angles, large facets and long rhabdoms; and (3) a yellow dorsal fovea with extremely small interommatidial angles, very large facets, and very long rhabdoms.

Measurements of interommatidial angle and facet diameter along vertical lines running frontally or laterally gave results very similar to the intermediate line described above, demonstrating that the main regional variations in the eye of *Sympetrum* are in the vertical direction. Because the visual field of the dorsal fovea is about circular and centered around the vertical axis, there is a binocular overlap involving the entire fovea.

The fovea covers only a small part (10°) of the eye's visual field, but the small interommatidial angles and the large facets make it expensive in terms of space. Observations of the pseudopupil reveal that the fovea, despite its small visual field, occupies a substantial part of the eye's surface. To illustrate this, the values plotted against vertical angle in Fig. 2a–c were also plotted against distance on the eye surface in Fig. 2d–f. Along the analysed vertical line the three eye regions each occupy about the same distance on the surface of the eye, despite the fact that the ventral region covers 67% of the vertical visual field, and the dorsal fovea only 5%.

The interommatidial angle and facet diameter can be used to calculate the optical radius of the eye (Table 1). For the dorsal fovea this gives an astonishing value of 11 mm. Thus, if the entire eye was built as the fovea the eye would have had a diameter of 22 mm, which falls in the middle of the size range of vertebrate eyes.

The ommatidial anatomy is that of a typical apposition eye (Armett-Kibel and Meinertzhagen 1983; Meyer and Labhart 1993). Apart from the pigmentation, the dorsal ommatidia differ in rhabdom arrangement from the ventral ones. At their distal tip, the dorsal rhabdoms are circular in cross-section, but only slightly below they become triangular. In most of the dorsal retina the triangular shape of the rhabdoms gradually changes into a three-lobed star (as seen in cross-section) of increasing arm length which contracts to a triangular shape again towards the proximal end of the rhabdom (see also Meyer and Labhart 1993). The total diameter of the lobed part of the rhabdoms is much larger than that of the unlobed distal and proximal segments. A similar arrangement was observed in the dorsal region of other dragonflies (Laughlin and McGinness 1978). In the dorsal fovea of *Sympetrum*, where the lobes are especially strongly developed, the arms of the star reach a maximal length of 7–8 μm at about one third of total rhabdom length. The rhabdoms of the dorso-frontal and ventral parts of the eye are not lobed at any level. As observed in the foveal part of the dorsal eye, the otherwise characteristic tiering of odonate retinulae is missing (Armett-Kibel and Meinertzhagen 1983; Meyer and Labhart 1993).

Table 1 Average eye radius, calculated as $D/\tan \Delta\phi$, for the three major eye regions, and the vertical field of view covered by these regions (D, lens diameter; $\Delta\phi$, interommatidial angle)

Eye region	Optical radius of eye (mm)	Vertical field of view (deg.)
Fovea	11	10
Yellow region except fovea	1.7	50
Ventral region	0.9	120

A feature characteristic for dragonflies is the presence of large tracheoles separating the ommatidia from each other all the way from the basement membrane to the layer of crystalline cones. The part of the eyes total volume that is taken up by the tracheoles is estimated to be more than 50%. An obvious reason for the presence of such voluminous tracheoles in the eye is for weight reduction of the large eyes (Laughlin and McGinness 1978) , but the anatomy does not exclude an optical function (see below).

Spectral sensitivity

The difference in screening pigment between the ventral and dorsal halves of the eye gives a clear indication that these dragonflies have spectrally different visual systems for vision above and below 30° elevation. This was confirmed by intracellular recordings: of the nine receptors from which we obtained stable recordings, seven were blue receptors (Fig. 3a), and two had maximum sensitivity in the near UV. Further evidence was given by ERG recordings, which peaked in the blue and UV, but showed no sensitivity above 520 nm (Fig. 3b). In contrast, the ERG of the ventral part of the eye showed sensitivity throughout the spectrum, with maximum sensitivity at long wavelengths (Fig. 3c). This agrees with the finding of UV-, blue-, green- and red-receptors in the ventral part of the eye of a related species,

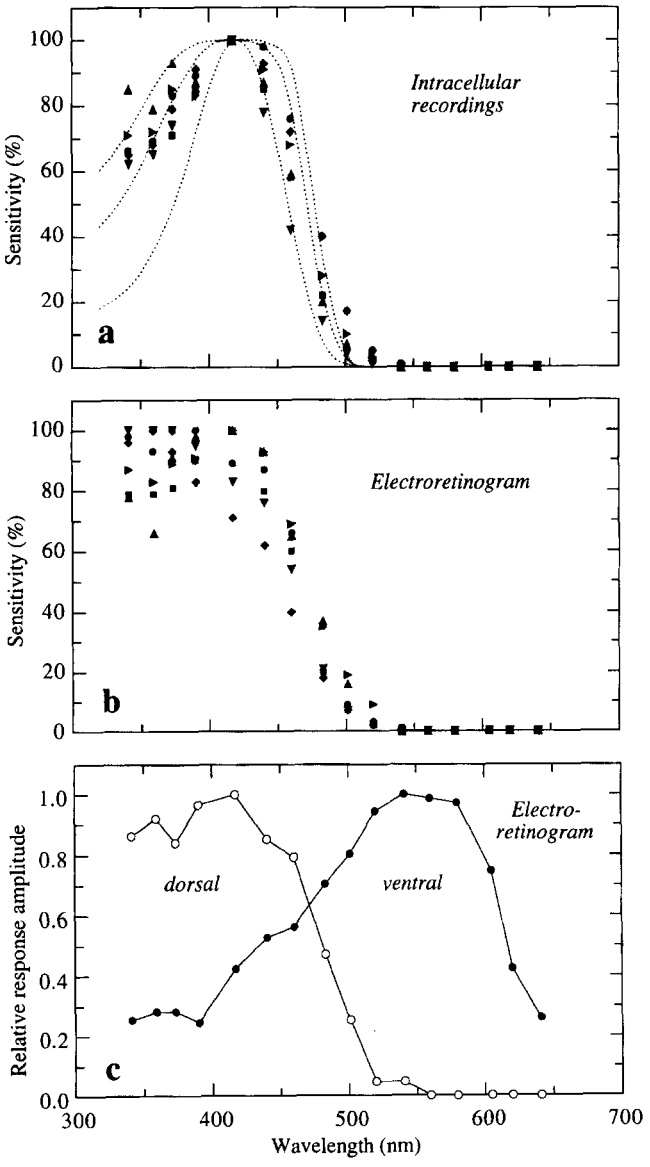

Fig. 3a–c Spectral sensitivity of the eye. **a** Results from intracellular recordings of blue receptors in the dorsal fovea. Different *symbols* represent data from 7 receptors. The *lower curve* is a Ebrey-Honig template for a 420 nm rhodopsin; the two *upper curves* are expected spectral sensitivities of receptors with a rhabdom length of 600 μm and 800 μm, respectively (see Fig. 10b). **b** Spectral sensitivity of the dorsal fovea, recorded by ERG. Different *symbols* represent data of 6 preparations. **c** Spectral efficiency of the dorsal fovea and of the ventral part of the eye (ERG recordings of one animal). In the ventral part of the eye the ERG response to the stimulus flash exhibits a complex, multiphasic shape varying with stimulus wavelength: the signal component of largest amplitude was taken as a measure of the response. – Spectral sensitivites indicate that the dorsal eye contains short wavelength receptors only

Sympetrum rubicundulum (Meinertzhagen et al. 1983). Similar spectral differences between dorsal and ventral eye have been reported in other libellulid species (Mazokin-Porshniakov 1959; Ruck 1965; Horridge 1969).

The spectral sensitivity functions of blue-receptors in the dorsal part suggest a rhodopsin with a sensitivity peak, λ_{max}, near 420 nm. This is supported by fitting rhodopsin templates (Bernard, personal communication) to the spectral sensitivity data of the blue-receptors. The quality of the two recordings of UV-cells was not good enough to specify λ_{max}. Although both UV- and blue-receptors are present in the dorsal part, there are several indications that at least the fovea is dominated by blue-cells: (1) blue-cells are more frequently impaled than UV-cells; (2) the contribution of UV-cells to the ERG comparatively small (Fig. 3b); (3) in microspectrophotometric preparations of foveal rhabdoms the UV-rhodopsin was not detectable (Schwemer, personal communication).

The photopigment system

The visual pigment system of the fovea was investigated in vivo, using the ERG response as a probe (compare Hamdorf et al. 1971). After adapting the eye with intense blue light (BG 12) for 10–30 s the ERG response was strongly reduced. This was only partly an effect of light-adaptation, because 3–4 min after the blue illumination the response had recovered to a steady-state level which was considerably below the original dark-adapted response level. The original response level could, however, be restored by illumination with yellow light (Fig. 4a). The speed of recovery increased with the intensity of yellow illumination (compare Fig. 4a and Fig. 4b). The same phenomena were observed with intracellular recordings from blue receptors in the dorsal fovea. The response recovery following strong blue illumination is presumably due to recovery of transduction gain, and to the action of a pupil mechanism (see Warrant and Pinter 1990). The recovery caused by yellow illumination, on the other hand, must be the result of a photochemical effect. The sensitivity of insect photoreceptors is correlated with the concentration of rhodopsin in the rhabdom (Hamdorf et al. 1973). Our observations thus indicate that blue light converts the blue-absorbing rhodopsin into a yellow-absorbing metarhodopsin ($R \rightarrow M$), which in turn is converted back to rhodopsin ($M \rightarrow R$) by yellow light.

The final level, to which the response recovers after blue illumination, depends on the amount of yellow light delivered to the eye. Thus, using strong yellow flashes of variable quantum content, intermediate levels of recovery could be obtained (Fig. 4c, d). In stable preparations the relation between yellow irradiation and response recovery could be quantified by monitoring the amplitude of the ERG to weak blue test flashes, first after "full blue adaptation", i.e. a blue flash strong enough to produce the maximum possible sustained reduction in response amplitude, and then giving "variable yellow irradiation" of known quantal content. A final yellow flash, "full yellow adaptation", strong

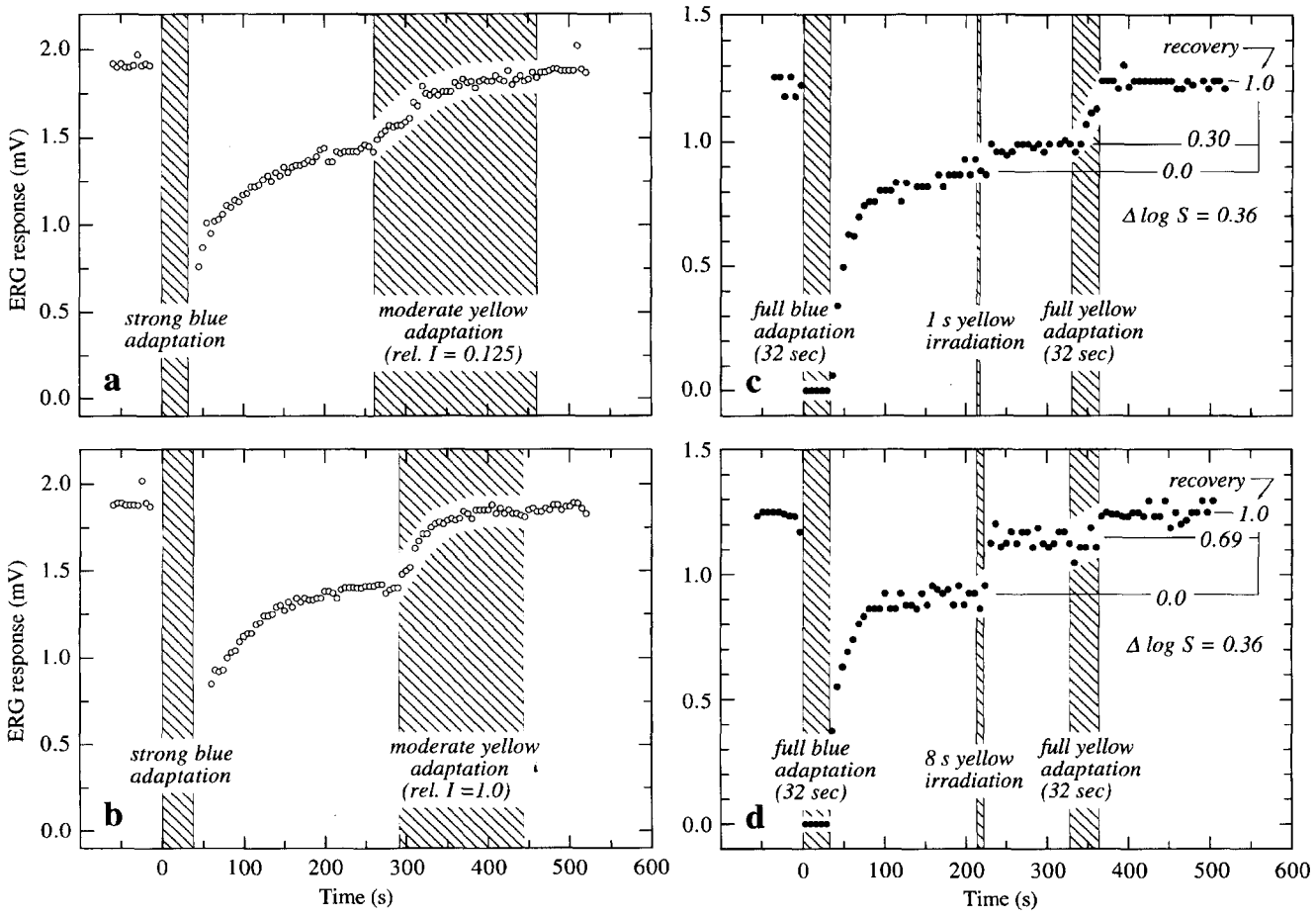

Fig. 4a–d Recovery of sensitivity by yellow adaptation in the blue adapted retina. In vivo photochemistry studied by the ERG. After strong blue adaptation (BG12 filter) the ERG response recovers only partly in the dark, but is fully recovered by irradiation with yellow light (OG550 filter). The ERG responses that were used to probe sensitivity of the eye (*symbols*) were elicited by weak flashes of blue light. **a, b** Recovery of the response by continuous yellow light of moderate intensity. Note the different rate of recovery depending on the intensity of yellow irradiation (**a** vs. **b**, relative intensities 1:8). **c, d** After *full blue adaptation* the response is recovered by strong, short flashes of yellow light (*yellow irradiation*). Note the different levels of recovery depending on the amount of quanta (controlled by flash duration) of yellow light (**c** vs. **d**, relative quanta 1:8). A second yellow flash of long duration serves to recover the response completely (*full yellow adaptation*). This type of experiment was used to quantify response recovery as a function of the amount of yellow quanta delivered to the eye. – Data indicate presence of a yellow-absorbing metharhodopsin

enough to fully restore the original response-amplitude, completed the experiment. The fraction of sustained response-recovery after variable yellow irradiation, compared with the complete response range between full blue and full yellow adaptation, was taken as a quantitative measure of response recovery (see Fig. 4c, d). The values of sustained response recovery were plotted versus log relative quanta of yellow irradiation, and could be fitted with a linear regression (Fig. 5). Thus, the intensity characteristic of response recovery (recovery function) shows an approximately linear relation to the log number of quanta delivered during the variable yellow flash. The sensitivity difference between fully yellow and fully blue adapted eyes was 0.36 ± 0.03 log units as measured in 6 animals using the ERG. Reliable estimates were less easily obtained with intracellular recordings giving values of c. 0.4 log units.

The spectral absorption properties of metarhodopsin were investigated by measuring the spectral efficiency of response recovery after isoquantal light flashes of different wavelengths[1]. The spectral sensitivity of response recovery was then calculated using the recovery function of the same preparation for calibration. Figure 6 shows the combined results of two successful preparations. Average spectral sensitivity of recovery peaks at 530–540 nm, giving a first estimate of the absorbance maximum of metarhodopsin. Using template absorbance curves for visual pigments, the λ_{max} of

[1] The same experimental protocol as for measuring recovery functions was used, except for the step "variable yellow irradiation" which was replaced by "variable wavelength"

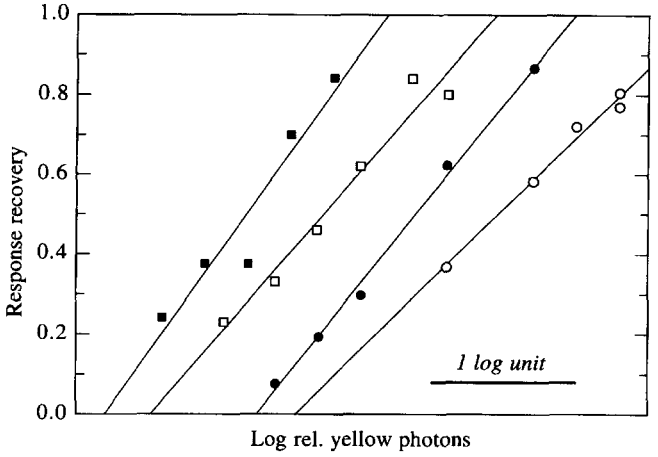

Fig. 5 Response recovery as a function of log yellow quanta delivered to the retina (recovery functions) of four preparations. In vivo photochemistry studied by ERG

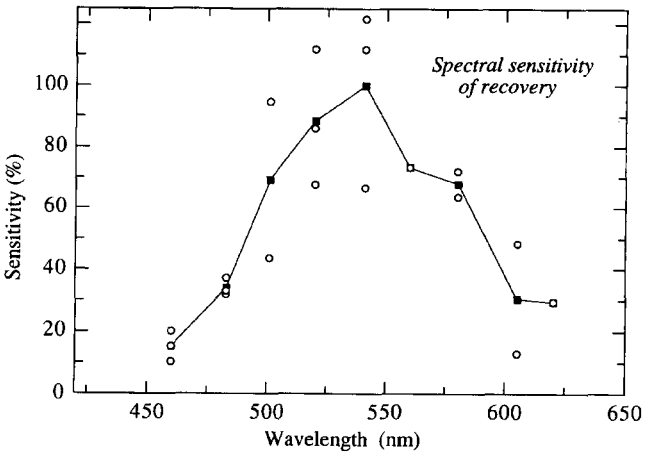

Fig. 6 Spectral sensitivity of response recovery. In vivo photochemistry studied by ERG. Combined data of two preparations (*empty circles*) and average sensitivity function (*line graph* and *filled squares*) are shown. The data indicate the presence of a metarhodopsin absorbing maximally between 530 and 540 nm

metarhodopsin can be calculated from these data: assuming $\lambda_{max} = 420$ nm for the rhodopsin, the λ_{max} of the metarhodopsin was adjusted such that the difference spectrum of the blue and the yellow adapted pigment system best fitted our data. This procedure confirms our estimate indicating a λ_{max} near 540 nm (Bernard, personal communication). Our results are supported by preliminary microspectrophotometric data, pointing to a metarhodopsin with a λ_{max} at 535 nm (Schwemer, personal communication).

The hypothesis that the dorsal screening pigment is yellow in order to improve reconversion of metarhodopsin requires that the screening pigment absorbs efficiently below 520 nm, where the rhodopsins are sensitive, but transmits some of the light above 520 nm that is exclusively captured by metarhodopsins. This was tested by microspectrophotometry. The distal parts of dorsal eyes were teased apart with needles on a microscope slide, and lumps of isolated yellow pigment were located for measurements. The results do indeed confirm the hypothesis: the screening pigment absorbs efficiently below 500 nm, but transmits above that wavelength (Fig. 7). Maximum transmission is at about 700 nm. The slope of the transmission curve is of course a function of the depth of the lumps of pigment in our preparations, and this is hard to relate to conditions in the intact eye. All of the measured lumps were rather large (similar in size to the crystalline cones which occasionally were also seen in the preparations), and we believe that the 50% transmission level located at 580 nm is reasonably accurate. The conclusion must thus be that light transmitted through the screening pigment has a considerable spectral overlap with the metarhodopsins in the dorsal eye-region, but hardly any with the rhodopsins (see Fig. 11a).

The consequence of this conclusion is that different optics apply to light used for vision and light used for reconversion of photopigment. In the following, we

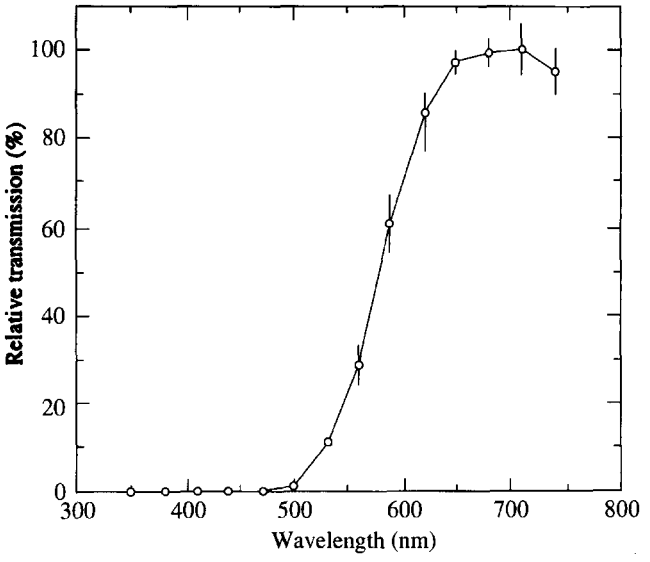

Fig. 7 Transmission of the yellow screening pigment in the dorsal region of the eye. The average from 14 measurements in 3 individuals is normalized to 100% (*vertical lines*, standard deviation)

investigate the details of the dorsal-eye optics in the two wavelength ranges.

Short wavelength visual optics

Intracellular recordings of angular sensitivity revealed acceptance angles, $\Delta\rho$, of $0.52° \pm 0.16$ (mean $\pm$ s.d., $n = 8$), with a smallest value of 0.33°. These values were obtained with a stimulus subtending 0.2°, which makes it likely that the average of 0.5° overestimates $\Delta\rho$ to some extent. Difficulties in locating the exact centre of these extremely narrow visual fields, and the limited

angular resolution of the perimeter (0.1°), makes the determination of $\Delta\rho$ only approximate. However, the measured values are in good agreement wthe acceptance angle calculated as follows from anatomical data. The facet diameter of the dorsal fovea is about 70 μm, and this would produce a diffraction blur spot subtending about 0.34° (λ/D radians, where λ is the wavelength, taken to 420 nm, and D is the facet diameter). The smallest angular sensitivities were between 0.3° and 0.5°, and they were from the fovea which apparently operates close to the diffraction limit. From anatomy we know that in the fovea the rhabdom diameter is about 1.5 μm, and the distance from inner corneal surface (a likely position of the nodal plane) to the distal rhabdom tip is about 305 μm. This gives the rhabdom an angular subtense in object space of 1.5/305 (rad), which amounts to 0.28°. Combining the rhabdom angular subtense with the diffraction blur (Snyder 1977), $\sqrt{(0.33^2 + 0.28^2)}$, we arrive at an estimated acceptance angle of 0.43°[2].

The presence of tracheoles separating the ommatidia has led to the idea that the entire retinula column, being surrounded by air, may act as an ommatidial light guide (Horridge 1969; Laughlin and McGinness 1978). We have found no evidence for such a mechanism. (1) Since the rhabdoms first expand and then taper only slowly along their length, it is likely that the light used for vision remains guided within the rhabdoms. (2) No light could be detected travelling outside the rhabdoms in our experiments on eye caps, described below (Fig. 9c). We conclude that the short wavelength visual optics of the *Sympetrum* dorsal eye fits predictions based on regular apposition principles.

Long wavelength reconversion optics

We now turn to the more complicated task of assessing the eyes optics for yellow light which the animal cannot see. Since no receptor potentials can be obtained from flashes of yellow light, we instead measured the angular efficiency of recovery, and calibrated the measurements against the recovery function. This procedure requires stable recordings for several hours, making ERG recordings the only possible approach. Like in our other ERG experiments, only a small circular spot of the dorsal fovea was exposed. The angular efficiency of recovery was recorded with isoquantal yellow (OG 550) flashes to induce recovery of the response to weak blue test flashes on axis. Both the blue and the yellow flashes had an angular subtense of 0.5°. The yellow flashes

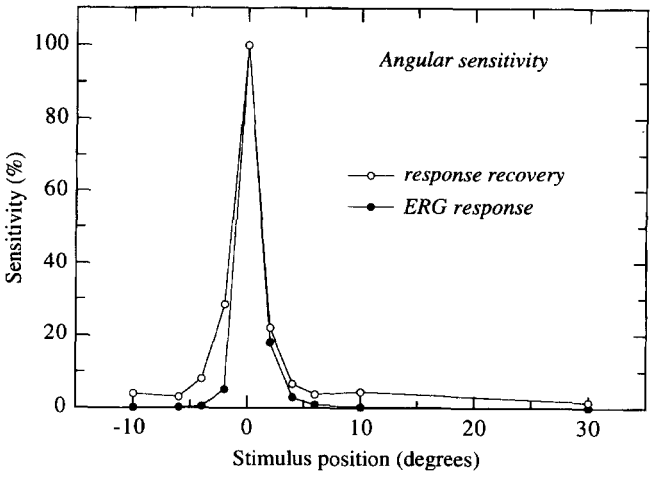

Fig. 8 Comparison of angular sensitivity functions for vision (blue light) and for metarhodopsin to rhodopsin reconversion (yellow light). The diagram shows the angular sensitivites of the ERG response (vision, *filled symbols*), and of response recovery (M − > R reconversion; *empty symbols*). Data were obtained from a circular spot of the fovea, 12 ommatidia in diameter, representing less than 4° of visual space; the rest of the eye was occluded. The results are from one of the three animals studied in this way. The wide shoulders of the angular recovery function are a result of yellow light leaking through the screening pigment

were given at variable angular positions centered around the direction of maximal sensitivity of the exposed eye spot[3]. The results were calibrated with a recovery function from the same preparation, to obtain the angular sensitivity of recovery (Fig. 8). On every preparation we also measured the conventional angular sensitivity function of the ERG response using blue test stimuli at different angular positions (Fig. 8). These two angular sensitivity functions thus represent the spatial acceptance profiles for yellow and blue light, respectively.

The blue acceptance functions are bell-shaped, having half-widths ranging from 1.7° to 2.7° in different ERG preparations. The yellow acceptance functions were of approximately similar shape and width. However, the blue acceptance functions drop to less than 1% between 5° and 10° off-axis, whereas the yellow acceptance functions have low-level flanks reaching at least as far as 30°–35° off-axis (Fig. 8). It seems likely that the central peak represents light being guided in the rhabdom. The flanks, however, cannot originate from light entering the rhabdom in the normal way. Note that blue light does not result in any flanks, implying that the flanks are caused by yellow light

[2] The calculation assumes a Gaussian blur circle and a Gaussian rhabdom profile. A slightly smaller acceptance angle would be expected with the real blur circle and the real waveguide-mode profile of the rhabdom (ven Hateren 1984)

[3] The same experimental protocol as for measuring recovery functions was used, except for the step "variable yellow irradiation" (at fixed, centered position) which was replaced by "isoquantal yellow at variable position"

transmitted through the screening pigment[4]. The large angular extent of the flanks makes diffuse spread in the screening pigment a likely explanation. The experiment demonstrates that yellow light transmitted through the screening pigment is indeed effective in reconverting metarhodopsin to rhodopsin.

Off-axial yellow acceptance is rather small compared to the center. But it has to be considered that the off-axial acceptance field occupies a much larger solid angle than the high-sensitivity center and will therefore contribute significantly to recovery under wide-field stimulation. In order to estimate the relative importance of the yellow light reaching the rhabdom through its distal tip and that leaking through the screening pigment, the volumes of the blue and yellow acceptance functions of the ERG preparations were calculated. Both sides of the functions were averaged, and rotated around the y-axis to generate three-dimensional functions for which the volumes were calculated. The volumes for yellow light from two preparations (for one of them see Fig. 8) were approximately 5 and 10 times larger than for blue light, respectively. Thus, for an extended source, the spot of 12–18 facets in diameter that was exposed in our ERG preparations would receive 4 to 9 times more yellow light because of transmission through the screening pigment. Under natural conditions, where all ommatidia are exposed, the gain in yellow light should be distinctly larger.

An optical way to test if long wavelengths are spread in the retina is to hemisect a fresh eye along the ommatidia (which is easily done with dragonfly eyes) and illuminate a small corneal patch, close to the cut, with light normal to the corneal surface. To do this experiment, we used a HeNe-laser because it provides the necessary parallellity and a sufficiently long wavelength. A small pin-hole close to the eye reduced the beam diameter to illuminate only a few facets. The outcome of this experiment (Fig. 9a) demonstrates a far-reaching diffuse spread in the retina.

Exactly where in the optical system does this light enter the retina? This can be answered using reversed illumination and the technique of corneal neutralization (Franceschini and Kirschfeld 1971) to observe light emerging from the ommatidial aperture. For this purpose we again used a hemisected eye, but now illuminated with white light from a fibre bundle which was placed such that light entered the exposed retina from the side. Focusing the microscope some 300 μm below the neutralized cornea, each facet displayed a small white dot surrounded by a large yellow disc (Fig. 9b). We interpret the white dot as unfiltered light emerging through the proximal tip of the crystalline cone, and the yellow disc as light transmitted through the yellow screening pigment around the sides of the crystalline cone. Because we observe a plane close to the focal plane of the corneal lens, the image we see is related to the angular sensitivity and reconversion functions. There is indeed a striking similarity with the angular reconversion function (Fig. 8): a sharp central peak surrounded by wide flanks. These observations thus provide evidence that the flanks on the angular reconversion function originate from off-axis light that enters the retina through the side of the crystalline cone, and then is scattered and filtered in the yellow pigment cells surrounding the cone.

To investigate the fate of this scattered yellow light, caps were cut off from intact eyes down to approximately half the retinal depth. Cutting across ommatidia is difficult in dragonflies because of the tracheoles running longitudinally. The success rate was still good enough to make a few reasonably undamaged caps, which were mounted as a hanging drop with the cornea in air. The preparations were then placed in a compound microscope, illuminated from the corneal side, and the cut surface photographed through a X10 objective. With a 410 nm interference filter in the beam path, isolated dots were visible (Fig. 9c). Due to the slightly disturbed surface it proved impossible to determine the exact diameter of the dots, but since they were very much smaller than the diameter of retinula cell columns, it must have been individual rhabdoms. This result demonstrates that blue light travels only within the rhabdom. Changing the filter to a 590 nm interference filter dramatically changed the result (Fig. 9d). The small bright dots were entirely gone, and light was evenly distributed over the cut retinal surface. This experiment shows that the retina is flooded with yellow light transmitted through the screening pigment. At first sight the result seems to contradict the sharp peak in the angular reconversion function, but we must note that the light guided by the rhabdom is attenuated by rhodopsins and metarhodopsins much faster than the unguided light. The preparation in Fig. 9d must therefore be at a depth in the eye where the unguided light dominates.

Our conclusions about the optics of yellow reconverting light is that on-axis light is guided by the rhabdom, just as blue visual light is. In addition yellow light enters the eye off-axis, and is scattered by the yellow screening pigment such that it floods the retina. There are no indications that the scattered yellow light is guided by rhabdoms nor by retinula columns. The large amounts of scattered long-wavelength light in the retina is the probable cause of the orange glow forming the pseudopupil of the dorsal eye.

Discussion

The dorsal part of the compound eye of *Sympetrum* dragonflies is specialized for detection of small objects

[4] A similar wavelength dependence of the optics was found in *Calliphora* by Streck (1972)

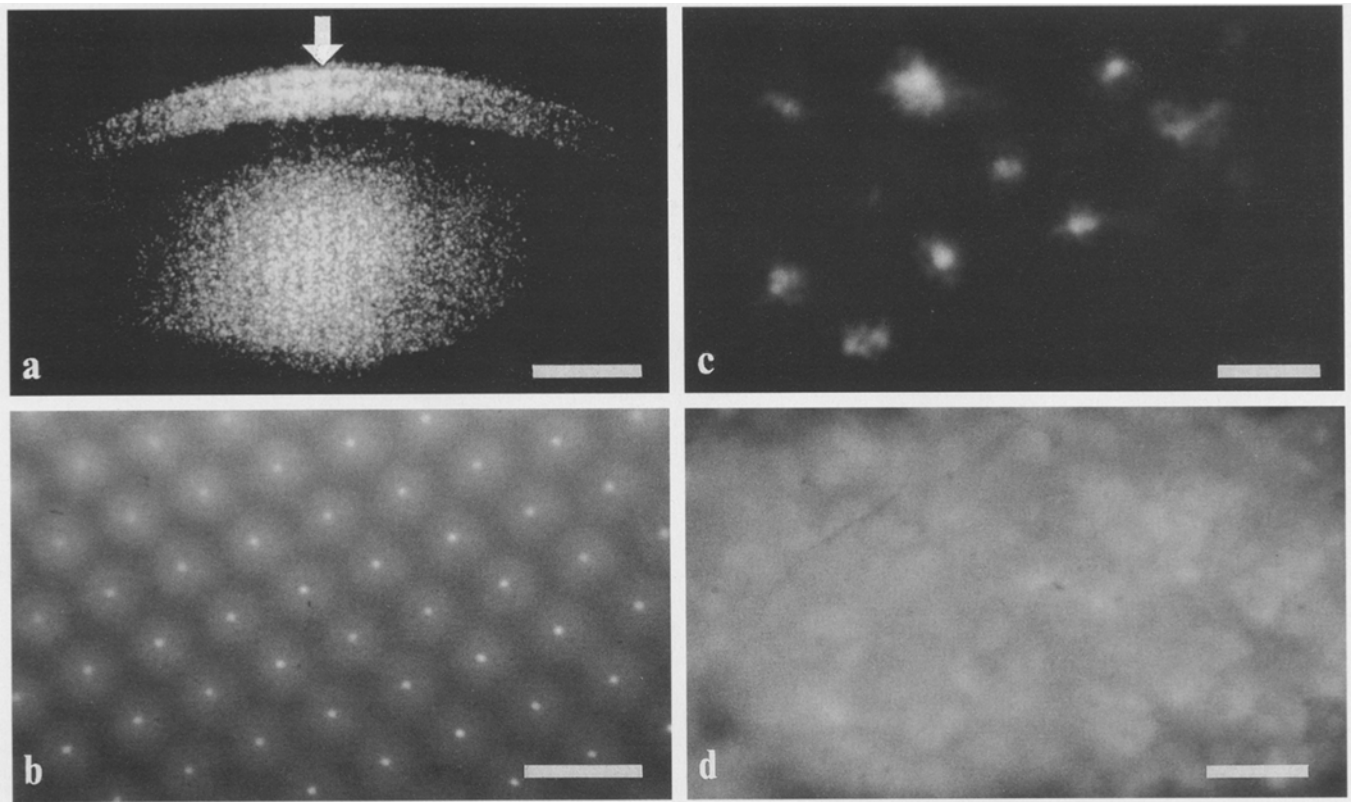

Fig. 9a–d Optical experiments illustrating the spread of yellow light in the dorsal eye. **a** A red laser beam enters through the cornea of a fresh eye which has been cut open along the ommatidia. The position and direction of the laser beam is indicated by the arrow (arrow-shaft width approximately corresponds to beam width). Scattered light from the originally parallel beam reveals an extensive spread of long-wavelength light in the retina. **b** Optically neutralized cornea with bright dots (distal rhabdom tips) surrounded by low intensity yellow discs. The white light which was injected sideways in the retina is transmitted through the yellow screening pigment, producing the yellow discs around the rhabdom tips. **c, d** An eye cap preparation observed at the cut surface in the retina, and illuminated from the corneal side with 410 nm blue light (**c**) and 590 nm yellow light (**d**). The blue light is confined to the rhabdoms whereas the yellow light evenly fills the retina. *Scale bars*: **a** 500 μm; **b** 100 μm; **c, d** 50 μm

against the blue sky. In this paper we have shown that the dorsal part of the eye contains a screening pigment which absorbs blue and ultraviolet light but transmits and scatters yellow light. The dorsal retina is sensitive only to blue and ultraviolet, and for these wavelengths the apposition ommatidia are optically isolated. Due to long-wavelength metarhodopsins the yellow light strongly favors photoreconversion of its visual pigment. Since the ommatidia are not optically isolated for yellow light, the eye collects light for reconversion of visual pigment much more efficiently than it collects light for vision. The enhanced catch of yellow light is possible because the animal is blind in this part of the spectrum, and the shielding, which is normally required for spatial resolution, is no longer necessary. The benefit of this arrangement is that the extra yellow light increases the reconversion rate of visual pigment, thus keeping rhodopsin concentrations much higher than otherwise would be possible.

The dorsal eye contains a fovea with extremely high spatial resolution: both interommatidial angles and acceptance angles approach 0.3°. The high resolution is of course expensive in terms of photon catch, and it makes sense, therefore, to implement special mechanisms in order to keep rhodopsin concentration high.

The principle of spectral separation

Invertebrates have bistable visual pigments. When a photon is absorbed by a rhodopsin molecule, a thermostable metarhodopsin is produced, and this is eventually reconverted back to the original rhodopsin state by the absorption of yet another photon (see Hamdorf 1979; Stavenga and Schwemer 1984). Only the forward reaction results in a transduced visual signal, and reconversion serves solely to restore functional visual pigment. With a constant spectral composition of the incident light, a steady state rapidly develops, and the rhodopsin concentration remains constant even when intensity changes. This system may at a first glance appear superior to the comparatively slow enzymatic pathway for reactivating the visual pigment of vertebrates, which is also present as an alternative route in insects (see e.g. Bernard 1983;

Schwemer 1993). The advantage is illusive, however, because invertebrates will have to waste precious photons on the reconversion process, whereas vertebrates can, theoretically at least, turn every available photon into a transduced visual signal. At steady state an invertebrate will have to absorb the same number of photons for reconversion as it absorbs for vision. This means that exactly half the number of photons that are available to an invertebrate photoreceptor must be wasted in order for it to see the other half. This competition for photons can be minimized if the spectral absorbance curve of the metarhodopsin is shifted relative to that of the rhodopsin. Generally, rhodopsins with absorbance maxima below 510 nm have metarhodopsins bathochromically shifted to longer wavelengths. Rhodopsins peaking above 510 nm instead have metarhodopsins hypsochromically shifted to shorter wavelengths (see Stavenga 1989).

As already concluded, the yellow screening pigment in the dorsal eye of *Sympetrum* is a specialization which allows for a more efficient photorecoversion of the visual pigment. The photopigments of the dorsal eye are sensitive to blue and ultraviolet, and as expected, the metarhodopsins are shifted to longer wavelengths. In combination with screening pigments that transmit long wavelengths, this makes a clever system where the optics for vision can be designed for high spatial resolution, but the optics for reconversion can be much more generous in admitting photons into the photoreceptors. This way of spectrally separating the optics for vision and reconversion can only be used in the dorsal part of the eye. In the ventral part, the additional presence of long wavelength rhodopsins require screening pigments that absorb all wavelengths.

As pointed out in the Introduction, this "spectral separation" of the optics has been demonstrated or indicated in a number of insects with specialized dorsal eye regions (Horridge 1976; Kirschfeld and Wenk 1976; Schneider et al. 1978; Stavenga 1979; Horret al. 1982; Menzel et al. 1991; Stavenga 1993; for review see Stavenga 1992). In such dorsal eyes the UV-blue range is used for vision, and bathochromic metarhodopsins in combination with long-pass screening pigments are used for reconversion. It would in principle be possible to have a visual system sensitive to long wavelengths, and reconversion occurring with short wavelengths transmitted through a blue screening pigment. But there are several reasons favouring the existing type of system: (1) shorter wavelengths are potentially better for vision because of less diffraction blur; (2) the spectral absorbance functions of all known photopigments have a far reaching shoulder on the short wavelength side (Stavenga et al. 1993), and this would overlap with the blurred but efficient optics intended for a short wavelength metarhodopsin; (3) thermal isomerizations of rhodopsin (Barlow et al. 1993), which induce noise in a photoreceptor, increase with peak absorbance wavelength. On the basis of these arguments it would be utterly surprising to find a visual system spectrally reversed to that of *Sympetrum*.

The significance of rhabdom length

In the foveal part of the dorsal eye the rhabdoms exceed 1 mm in length, and to our knowledge these must be the longest photoreceptive elements in the animal kingdom. It is of course tempting to explain this as yet another way of improving sensitivity, but with the normally assumed extinction coefficients of invertebrate photoreceptors (0.0067/μm: Bruno et al. 1977), a rhabdom length of 687 μm is sufficient to absorb 99% of the incident light, and most insect rhabdoms are much shorter than this (for equations see Kirschfeld 1974). With a length increase to 1.1 mm, only 0.94% more light is absorbed. Is less than 1% gain good enough to motivate almost a doubling of rhabdom length?

It is intriguing, in this respect, that *Sympetrum* and other dragonflies have rhabdoms which are more than twice as long as those of most other insects. Dragonflies are large insects, and therefore have the extra space available for long photoreceptors. There is, however, another line of argument based on the amount of light absorbed. The statement above that 99% of the incident light is absorbed a rhabdom of slightly less than 700 μm, is incorrect because it is based on an abuse of the extinction coefficient (Land 1981). The standard usage of the photoreceptor extinction-coefficient is the fraction of light absorbed per μm path length at *peak-absorbance wavelength*. Naturally, light of all other wavelengths is absorbed at a slower rate. Since photopigments in general have a broad bell-shaped spectral absorbance curve, the extinction coefficient for the peak of the curve cannot be applied to the whole curve. When 99% of the light at peak-absorbance wavelength has been absorbed, there is much more than 1% left within the entire spectral absorption range (Fig. 10a). This also means that the spectral sensitivity becomes broader with a longer photoreceptor (Fig. 10 b). The phenomenon is termed self-screening and has been well known for decades (see Hamdorf 1979). In order to calculate how much of the incident light that has been absorbed in a photoreceptor of given length it is necessary to compute the integral of the complete absorption curve. Performing this calculation (Fig. 10c), it is obvious that rhabdom lengths exceeding 1 mm are motivated for absorption reasons alone. For a rhabdom length of 1.1 mm, where the standard absorption coefficient predicts 99.94% absorption, the actual absorption is only 82%.

An interesting consequence of self-screening is that the rhabdom segment added beyond 700 μm hardly improves sensitivity to the peak wavelength of the visual pigment. Instead, a sensitivity gain is brought about by a widening of the spectral sensitivity curve. In

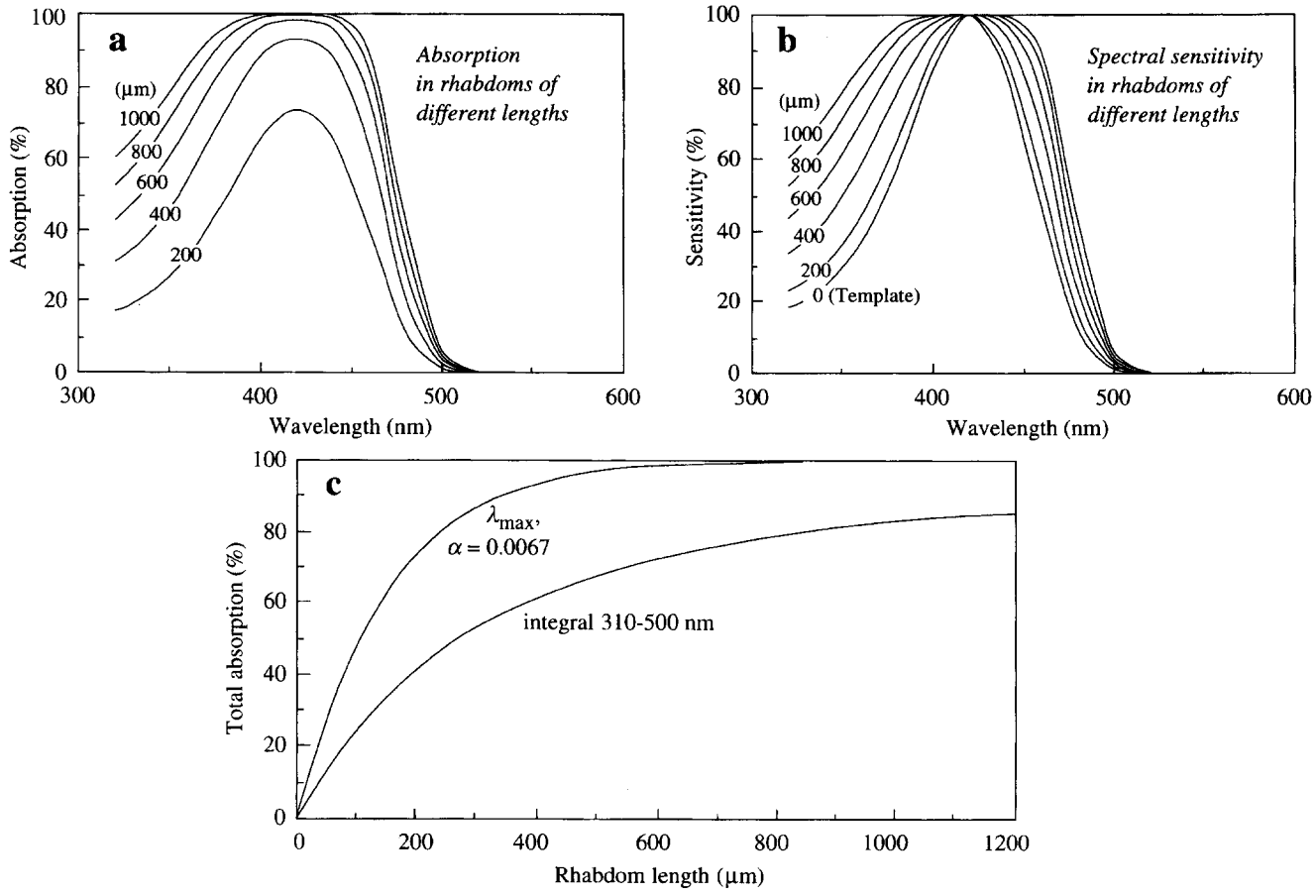

Fig. 10a–c Calculations of absorption in a cylinder (rhabdom) containing a 420 nm rhodopsin (template by Ebrey and Honig 1977). The extinction coefficient for 420 nm was taken to be 0.0067 μm^{-1}. **a** The absorption as a function of wavelength is shown for 5 different rhabdom lengths. **b** The same data as in **a**, normalized to 100% gives the spectral sensitivity which is expected if the rhabdom contains no other absorbing compounds. **c** Total absorption as a function of rhabdom length, calculated for the peak absorbance wavelength, λ_{max}, alone, and for the spectral window 310–500 nm. The latter function is the normalized sum of exponential extinction curves for all wavelengths in the spectral window

agreement with this line of thought, the measured spectral sensitivity functions of the blue receptors are considerably wider than the template of a 420 nm rhodopsin (Fig. 3a). The spectral sensitivity functions are, however, narrower than predicted for a 1100 μm rhabdom (compare Figs. 3a and 10b), and this is possibly a result of lateral filtering both by the UV-absorbing rhodopsin and the metarhodopsins. This effectively means that *Sympetrum* gains less from the long rhabdoms than suggested by the integral curve of Fig. 10c.

Sensitivity and saturation of transduction units

It is relevant here to ask what *Sympetrum* may gain from having rhodopsin concentrations boosted by the yellow light transmitted through the screening pigment. The concentration of rhodopsin depends on the relative amount of light absorbed by rhodopsin and metarhodopsin, and on the quantum efficiencies of conversion. A rigorous analysis thus requires detailed knowledge about the photopigment system, which is not yet available for *Sympetrum*. It also requires knowledge about the spectral composition of light at every level of the rhabdom. Although this beyond reach at present, it is possible to make some informative estimates. Using standard templates for spectral absorbance (Ebrey and Honig 1977), and the fact that the photosensitivity at peak absorbance wavelength of blue rhodopsins is usually somewhat lower than that of the corresponding metarhodopsins (Stavenga and Schwemer 1984), we made a model system (Fig. 11a). Spectral data for blue skies (Fig. 11b) were obtained from the literature (Henderson and Hodgkiss 1963; McFarland and Munz 1974). The product of the skylight function and the photosensitivity curves of rhodopsin (R) and metarhodopsin (M), respectively, produces two new functions, the areas of which are proportional to the conversion rates k_R, ($R \rightarrow M$), and k_M, ($M \rightarrow R$), respectively. The fraction of the photopigment which is in the rhodopsin state, f_R, can then be calculated by $f_R = k_M/(k_M + k_R)$, (Stavenga and Schwemer 1984). The result is that 65% to 68% of the photopigment would be in the rhodopsin state, if the spectral composition was like skylight throughout the rhabdom.

If we continue to ignore the filtering effects of rhodopsins and metarhodopsins (including those of the UV

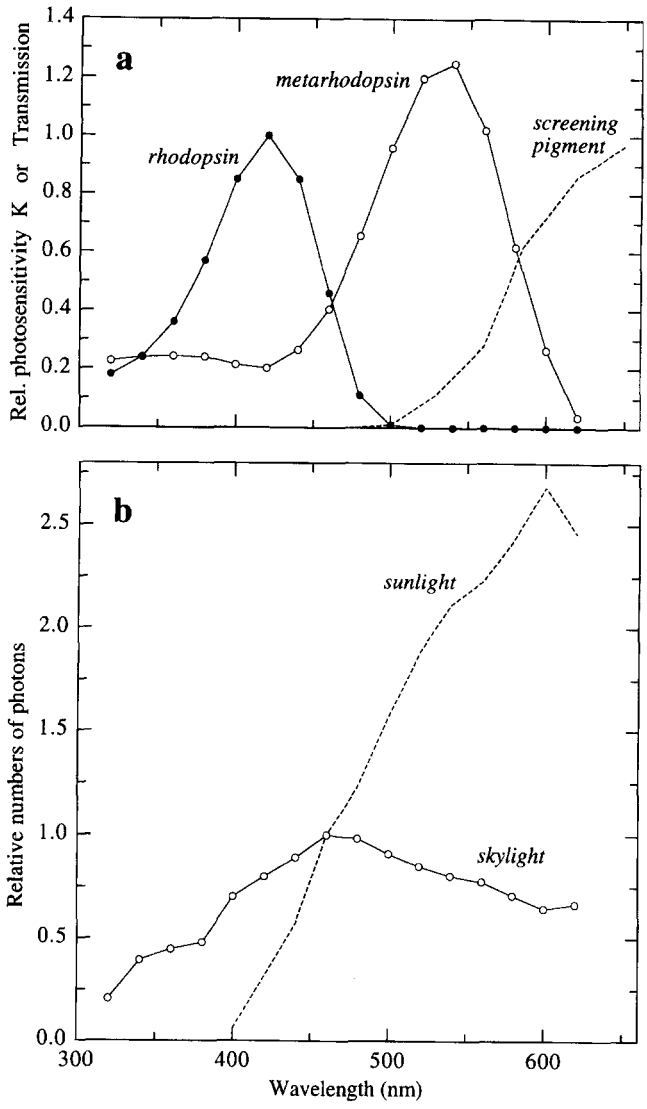

Fig. 11a, b Spectral absorption model of the dorsal eye (**a**), and the spectral composition of skylight (**b**) used for calculating rhodopsin concentration under natural conditions. The model system (**a**) contains a rhodopsin with $\lambda_{max} = 420$ nm and a metarhodopsin with $\lambda_{max} = 535$ nm (based on templates by Ebrey and Honig 1977) The relative difference in photosensitivity at λ_{max} for the rhodopsin and its metarhodopsin was taken to 1.25 (see Stavenga and Schwemer 1984 for common absorbance coefficients and quantum efficiencies of conversion). For comparison, the spectral transmission of the yellow screening pigment (from Fig. 7) is also indicated. The spectral composition (**b**) of blue skylight on a clear sunny day (exemplified by data from Henderson and Hodgkiss 1963) and of direct sunlight (both normalized at 460 nm). Conversion rates k_R and k_M are calculated from photosensitivities and radiance values at 20 nm intervals (*symbols* in a and b; compare text)

sensitive system), and just add the effect of yellow light transmitted through the screening pigment, we recall from our ERG experiments that the amount of light for reconversion ($M \rightarrow R$) was increased between 4 and 9 times. This implies that k_M increases by the same factor, and f_R then becomes 88%–95%. In our ERG experiments only a small spot of the dorsal fovea was exposed, whereas under natural conditions yellow light can enter through the entire dorsal part of the eye. We thus expect the yellow gain to be significantly higher than that obtained experimentally. Further, the amount of diffusely spread yellow light will make a manifold increase if the eye is in direct sunlight and not just exposed to a sunless blue sky as in the calculations above. This is because the direct sunlight, which contains mainly long wavelengths (see Fig. 11b), dominates total radiant energy from the sky (Gates 1980, p. 111 ff; own observations). *Sympetrum* is most active under full sunshine (Mayer 1961, own observations). This means that under natural conditions, the light leaking through the yellow screening pigment will pump virtually all photopigment into the rhodopsin state.

What are the consequences of the high rhodopsin concentrations for the eyes sensitivity? According to the arguments above, the yellow pigment increases the rhodopsin concentrations from c. 66% to almost 100% under natural conditions. In terms of total absorption, this is equivalent to an increase in rhabdom length by a factor of 100/66 = 1.52. Extending the integral curve of Fig. 10 to 1672 μm reveals a total absorption gain of only a few percent. From our discussions so far this seems to be the net gain brought about by the yellow screening pigment. Are a few percent higher sensitivity really enough to motivate a special screening pigment in the dorsal part of the eye?

Without answering this question we proceed with discussion of the signal to noise ratio in the photoreceptor cells. At daylight intensities the number of transduction units in the rhabdom imposes an upper limit to the signal to noise ratio. The reason is that at high intensities there are simply not enough transduction units to turn every absorbed photon into a voltage change across the membrane (Howard and Snyder 1983; Howard et al. 1987). Measurements in locusts and flies indicate that the signal to noise ratio starts to saturate between 10^6 and 10^7 quanta/s absorbed by a photoreceptor (Howard and Snyder 1983; Howard et al. 1987; Laughlin et al. 1987). It is thus relevant to estimate the number of blue photons entering rhabdoms in the dorsal eye of *Sympetrum* under natural conditions. For this purpose we measured the radiance of the blue sky on three sunny days using a calibrated photodiode equipped with a wide-band blue filter and a diaphragm restricting the measured field to a diameter of 15°. From these measurements spectral radiance was calculated. We found radiance at 420 nm to vary between $1.3 \cdot 10^{13}$ and $3.9 \cdot 10^{13}$ quanta/s cm^2 sr nm, in the blue upper part of the sky, well clear of the sun. This means that within the spectral window 360–460 nm, $3.0 \cdot 10^6$ to $9.3 \cdot 10^6$ quanta/s enter the rhabdom of a dorsal ommatidium (lens diameter D, 70 μm; acceptance angle $\Delta\rho$, 0.4°; for calculation procedures see Dubs et al. 1981). Thus, even from the darker parts of the sky, each ommatidium receives at

least $3 \cdot 10^6$ quanta/s in the spectral range where the blue rhodopsin absorbs best. This estimate is only approximate, but it serves to show that the dorsal part of the *Sympetrum* eye receives enough light to risk saturation of transduction units.

If the transduction units are evenly distributed along the rhabdom it is clearly the distal part of the rhabdom that runs the greatest risk of saturation. Since saturation occurs when there are more rhodopsin molecules converted than the transduction units can cope with, the problem would ideally be solved by reducing the rhodopsin concentration in those parts of the rhabdom where the photon flux is highest. This implies that the signal to noise ratio would be improved if the rhodopsin concentration was low in the distal part of the rhabdom, and increased proximally. In the dorsal eye of *Sympetrum* the rhabdoms are narrow in the distal part, and they will thus be rather inefficient in picking up the diffusely spread yellow light. More proximally, however, the rhabdoms widen, and extensive lobes are formed, thereby facilitating the absorption of the yellow light transmitted through the screening pigment. The result is that rhodopsin concentrations can, in fact, be expected to increase proximally in the rhabdom. In addition, the number of microvilli per unit length of the rhabdom increases in proportion with the size of the lobes. Adopting the view that the microvilli represent the transduction units of invertebrate photoreceptors (Howard et al. 1987; Hochstrate and Hamdorf 1990), another effect of the lobes may be to increase the number of transduction units. It thus seems that the yellow screening pigment and the lobes of the rhabdom may be explained as adaptations improving the signal to noise ratio, which is of utmost importance for detecting small, fast objects.

The dorsal ommatidia of the dragonfly *Hemicordulia* are almost identical in size and structure to those of *Sympetrum* (Laughlin and McGinness 1978; Meyer and Labhart 1993). Laughlin (1989) has compared the signal quality of the photoreceptors of *Hemicordulia* and of the fly *Lucilia*, which has short, cylindrical rhabdoms. He found that the maximal signal to noise ratio that can be attained is indeed much higher in the dragonfly.

Functional regionalizations for targeting against the blue sky

From the external appearance the eye of *Sympetrum* seems to be divided into a yellow dorsal part for vision against the sky, and a normally pigmented ventral part for vision near and below the horizon. Further analysis, however, reveals a more complicated regionalization. The two most important parameters describing optical performance of an eye are resolution and sensitivity. The resolution, given as the sampling frequency, and the sensitivity to an extended source can both be calculated from the measured values in Fig. 2 (see Land 1981). For the sensitivity calculations we have to rely on the assumption, based on the theoretical optimization of the ommatidial mosaic, that the angular subtense of the rhabdom is roughly equal to the interommatidial angle: (Howard and Snyder 1983). Since the only significant regional variations occur along the vertical axis, it is most relevant to consider the variation of optical performance in the vertical plane (Fig. 12). It then becomes immediately obvious that the eye can be divided into three functionally distinct regions: ventral, dorsal, and dorsal fovea[5]. The ventral region is very uniform with a moderate sampling frequency, and an anatomical sensitivity typical for diurnal activity (see Land 1981; Nilsson and Modlin 1994). The fovea, on the other hand, displays an extremely high sampling frequency, but a comparatively low sensitivity. Intriguing values are found in the extra-foveal dorsal region where the sampling frequency is similar to that of the ventral region, but the sensitivity is surprisingly high, in fact more than a log unit better than in the fovea. Note that the sensitivity values plotted in Fig. 12 do not take the effects of scattered yellow light into account. It is thus likely that the yellow region, including the fovea, has a slightly better sensitivity than that indicated by the graph in Fig. 12. From electrophysiology we also know that the ventral region covers a large spectral-sensitivity range from UV to yellow, whereas the entire dorsal part is sensitive only to UV and blue.

We now have the basic information necessary for interpretations of the functional roles that the three eye regions may have. Both the spectral properties and optical design parameters are standard in the ventral part of the eye, indicating regular navigational functions. It is the dorsal half of the eye that displays all the specializations serving the characteristic behaviour of *Sympetrum*. Dragonflies of this genus spend most of their time on the ground. They seem to prefer spots on the ground with little or no vegetation. Reliable places to find them are on paths or other exposed areas on dry meadows. It appears that they patiently wait until a fly, or other insect prey, enters the dorsal visual field. When a suitable flying prey has been detected against the sky, the dragonfly rapidly takes off to pursue and catch it (Demoll 1913; Mayer 1961; own observations). The yellow dorsal part of the eye, with its short wavelength sensitivity, is undeniably well suited for targeting against the sky. Most of the dorsal visual field is covered by the extra-foveal dorsal region, and this is characterized by moderate resolution but very high

[5] We disregard here a fourth eye region, the extremely narrow dorsal rim area, which is specialized for polarization vision (Meyer and Labhart 1993)

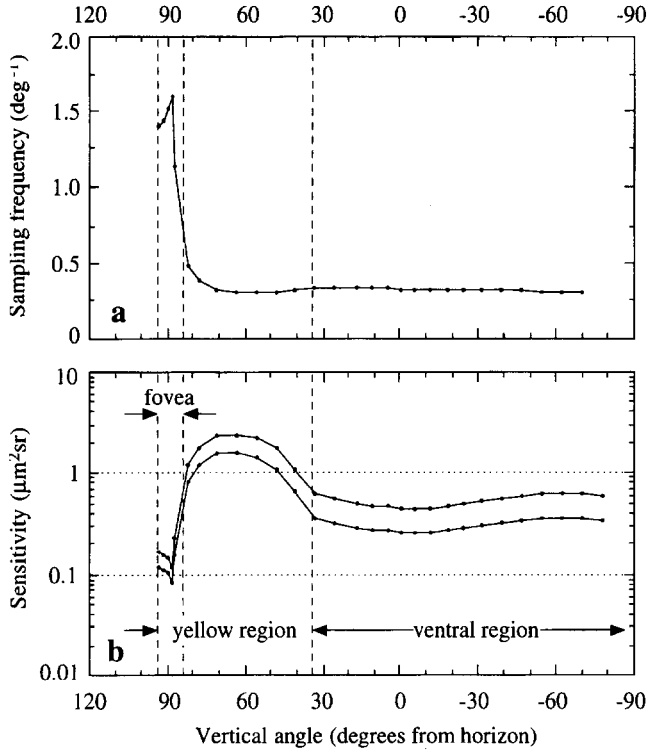

Fig. 12a, b The variation of resolution and sensitivity in the vertical plane, calculated from the data in Fig. 2. **a** The sampling frequency, $1/(\sqrt{3}\Delta\Phi)$ (for theory see Snyder 1979), shows that the fovea differs dramatically from the rest of the eye. **b** The calculated sensitivity (Kirschfeld 1974; Land 1981) reveals low values for the fovea, comparatively high values for the extrafoveal yellow region, and intermediate values for the ventral region. The angular subtense of the rhabdom, which is unknown in most of the eye, was taken to equal the local interommatidial angle. The upper sensitivity-curve is based on the assumed extinction coefficient at λ_{max}, 0.0067 μm^{-1} (Bruno et al. 1977), whereas the lower sensitivity-curve is based on the integrated extinction in the spectral window 310–500 nm (Fig. 10c)

sensitivity (Fig. 12). Assuming uniform sky intensity, extrafoveal dorsal rhabdoms will receive more than 10 times as much light as foveal rhabdoms. One reason for such a difference would be a corresponding difference in temporal resolution, specializing the extra-foveal dorsal region for detection of fast flying objects against the sky. Another way to exploit the high extrafoveal sensitivity would be an increased contrast sensitivity for detecting distant objects subtending only a fraction of the visual field of an ommatidium (Vallet and Coles 1991). Possibly both strategies play a role in facilitating prey detection by the extrafoveal yellow region. After spotting a potential prey, the fovea becomes important because of its extreme spatial resolution which is ideal for evaluation of prey suitability, and later, for tracking as the dragonfly approaches the prey from below. The large head movements that are often seen immediately before take off, support this interpretation of the division of labour between the foveal and extra-foveal parts of the dorsal area.

Two characteristic features of the dorsal area, the unusual size and shape of the rhabdoms and the spectral tuning of visual and screening pigments, are adaptations maximizing sensitivity and signal to noise ratio. We conclude that the entire dorsal region owes its colour and deep retina to design principles favouring prey detection against the blue sky.

Acknowledgements We are very grateful to Dr. Joachim Schwemer for dedicating considerable time to perform a number of microspectrophotometric measurements for us, and to Dr. Gary D. Bernard for his interest in the study and his help with fitting rhodopsin templates to our data. Kirsten Keller provided technical assistance with histology. We also thank Dr. Doekele Stavenga and Dr. Eric Warrant for critical comments of the manuscript. The project was supported by the Swiss National Science Foundation (grant 3.644-8.87) and the Swedish Natural Science Research Council (grant 01742-308).

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