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REVIEW

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Colour in the eyes of insects

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Abstract Many insect species have darkly coloured eyes, but distinct colours or patterns are frequently featured. A number of exemplary cases of flies and butterflies are discussed to illustrate our present knowledge of the physical basis of eye colours, their functional background, and the implications for insect colour vision. The screening pigments in the pigment cells commonly determine the eye colour. The red screening pigments of fly eyes and the dorsal eye regions of dragonflies allow stray light to photochemically restore photoconverted visual pigments. A similar role is played by yellow pigment granules inside the photoreceptor cells which function as a light-controlling pupil. Most insect eyes contain black screening pigments which prevent stray light to produce background noise in the photoreceptors. The eyes of tabanid flies are marked by strong metallic colours, due to multilayers in the corneal facet lenses. The corneal multilayers in the gold-green eyes of the deer fly *Chrysops relictus* reduce the lens transmission in the orange-green, thus narrowing the sensitivity spectrum of photoreceptors having a green absorbing rhodopsin. The tapetum in the eyes of butterflies probably enhances the spectral sensitivity of proximal long-wavelength photoreceptors. Pigment granules lining the rhabdom fine-tune the sensitivity spectra.

Keywords Colour vision · Screening pigment · Pupil · Spectral filters · Heterogeneity

Introduction

Insects obtain visual information about their environment via their compound eyes. These light organs provide the input for the visual neuropiles, which process the light signals to detect motion, colours, or patterns of interest. The numerous ommatidia of an insect eye collaborate to collect maximal optical information. Each ommatidium is composed of a facet lens, a (crystalline) cone, a set of photoreceptors and surrounding pigment cells (for structural details, see e.g. Nilsson 1989). In this review I will survey how the pigments of insect eyes affect both the appearance and the function of the eyes, i.e. determine the eyes' colour and modify their light sensitivity, respectively. I will restrict myself to apposition-type eyes and specifically treat a few exemplary cases, namely flies and butterflies.

The primary place of photodetection is the rhabdomere, a cylindrical structure of the photoreceptors, where the light absorbed by the visual pigment molecules is transduced into a neural signal. In flies, the eight photoreceptors of an ommatidium form an open rhabdom, i.e. the rhabdomeres remain separate, and each fly rhabdomere acts as an optical waveguide (Hardie 1986). In bees and butterflies, the nine photoreceptors of an ommatidium have their rhabdomeres joined into a fused rhabdom, which acts as one, efficient optical waveguide (review by van Hateren 1989).

Each ommatidium has its own integrated optical system, i.e. a facet lens and cone together with the light-guiding rhabdom(eres). The assembly of ommatidia usually provides the insect with excellent spatial resolution, at least given the small size of the eye (for an excellent and comprehensive review see Warrant and McIntyre 1993). The visual pigments of the photoreceptor cells determine the spectral sensitivity. With a set of spectrally different photoreceptor types colour vision is possible. I will discuss below how ocular pigments can influence the imaging and spectral aspects of insect eyes and thus their spatial acuity and spectral discrimination (see also Stavenga 1979, 1989, 1992).

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The screening pigments of fly eyes are red to increase photoregeneration of rhodopsin

The eye of the fruitfly is presently the most intensively investigated insect eye. The normal, wild type eye is marked by its bright red colour, which is due to a mixture of ommochrome and pteridine pigments concentrated in the screening pigment cells (review by Summers et al. 1982). A similar, rather dark red pigmentation is found in the eyes of all higher dipterans (Langer 1975; Summers et al. 1982).

The red screening pigment has a special function for fly vision (Stavenga et al. 1973), initially recognized in a study of the visual pigment of a blowfly appropriately named *Calliphora erythrocephala* (red head), now renamed *C. vicina* (Fig. 1a). The principal visual pigment, that of the photoreceptors R1–6, is a rhodopsin (R490) with a main absorption band in the blue-green (Fig. 1b). Light absorption converts it into a metarhodopsin (M570), which absorbs mainly in the orange. The metarhodopsin is thermostable, but can be photoconverted back into the original rhodopsin state (Fig. 1b). The ratio of visual pigment molecules existing in the rhodopsin state to those in the metarhodopsin state consequently depends on the spectral distribution of the illumination. For example, prolonged monochromatic blue light results in a low rhodopsin content of ca. 30%, red light favours a virtually 100% rhodopsin fraction, and broad-band, white light causes roughly 65% rhodopsin (Stavenga et al. 1973; Stavenga 1980).

The eyes of a fly in a natural situation receive broad-band sky and sun light from various directions. Light entering a facet lens on-axis is focused into the rhabdomeres, but off-axis light hits the screening pigments (Fig. 1b, inset). The long-wavelength part of it will pass the red-transmittant screening pigment and penetrate the eye. The red stray light thus can reach the rhabdomeres from oblique directions and thus can convert metarhodopsin molecules to their native rhodopsin state. This is easily demonstrated experimentally (Stavenga et al. 1973).

The early recognition of the role of the red screening pigment in fly eyes established the concept of the spectral tuning of insect retinal pigments (review by Stavenga 1992). The principle of tuned pigments, although generally accepted now as common knowledge, has received little critical attention, however. It is important to realize that severe constraints have to be met for it to work properly. An insight into the potential problems can be gained by comparing the angular sensitivity curves of photoreceptor cells of the wild-type blowfly *Calliphora* with those of its mutant *chalky*, which lacks screening pigment (Streck 1972). The angular sensitivity consists of two components, one central component, due to light reaching the photoreceptor more or less on-axis via its ommatidial facet lens, and a second, plateau component, due to off-axis light entering through other facet lenses (see Fig. 1b, inset). The central component is more or less gaussian shaped, and the plateau component is more or less flat. In the mutant *chalky*, the magnitude of the plateau appears to be about 4% of the sensitivity in the central peak. In wild type photoreceptors, the plateau is negligible at all wavelengths in the main, visible range, but it becomes similar to that of the *chalky* at red wavelengths (Streck 1972).

A simple calculation shows that the high plateau value hopelessly impairs the mutant's spatial vision in natural conditions. The relative contributions of the sky

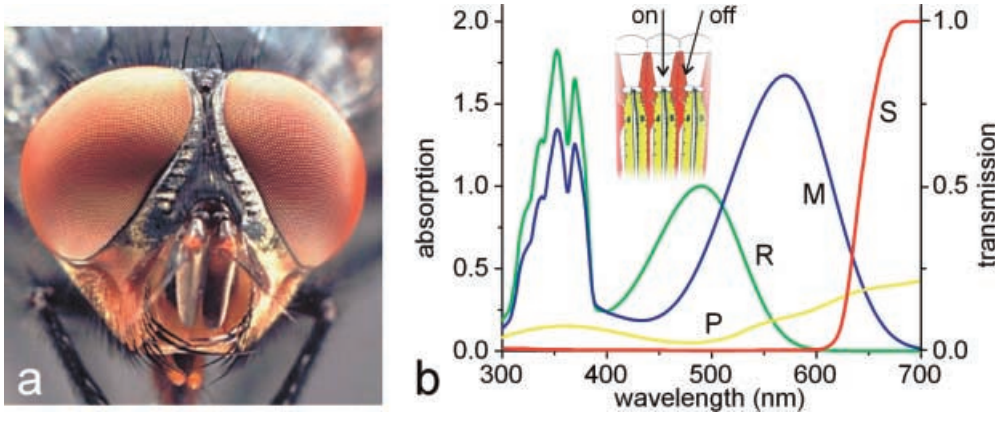

Fig. 1. The blowfly *Calliphora vicina* (**a** male) has red eyes due to red-reflecting screening pigment located in the pigment cells (**b** inset, red). The visual pigment, contained in the rhabdomeres of the main photoreceptors, R1–6, is a rhodopsin (*R*) absorbing maximally at about 490 nm. Its photoproduct, metarhodopsin (*M*), peaks at about 570 nm. A UV-absorbing sensitizing pigment enhances the photosensitivity of both visual pigment states (Minke and Kirschfeld 1979; Hardie 1986). The spectra are normalized at the rhodopsin absorption peak in the blue-green. The photoreceptor cells (**b** inset, yellow) contain pupillary pigment granules, which absorb predominantly in the blue and less in the yellow and red wavelength ranges. Accordingly, the transmission is higher in the yellow and red (*P*); the degree of pupil closure, which depends on the state of light adaptation, determines the actual magnitude of the transmission. The transmission of the red screening pigment (*S*) is negligible at wavelengths up to 600 nm, but rises rapidly above this value. Whereas the pupillary pigment filters light which has entered the facet lens on-axis and propagates along the rhabdomeres, the screening pigment filters light entering the facet lenses off-axis (**b** inset). Yellow and red light, remaining from white light filtered by the pupillary and the screening pigments, preferentially converts metarhodopsin into rhodopsin

and sun depend of course on the circumstances, such as whether or not the animal lives in an open field, or whether the sun is obscured by clouds or earthly objects, but both sources will always strongly activate the mutant's photoreceptors. The sky covers a spatial solid angle of 2π steradians, at least in the open field situation, which is in the order of 4 log units larger than that of the sun (angular diameter $\sim 0.5°$). The latter's ca. 4 log units higher brightness largely compensates for its small angle, so both stray light contributions can be similar. The field size of the sun is in the order of the visual field of a fly photoreceptor, and therefore any *chalky* photoreceptor will always be strongly stimulated, even when the sun shines from a severely off-axial direction. The response to light from the photoreceptor's central field will therefore be swamped by the few orders of magnitude more intense stray light, when screening pigment is absent. To keep the stray light noise component of the photoreceptor signal within bounds it is compulsory to install dense screening pigment, i.e. the optical density must be well over 3 log units in the visible range (Goldsmith 1965). On the other hand, if stray light will rapidly photoconvert existing metarhodopsin molecules, the screening pigment density has to be minor at those wavelengths where metarhodopsin substantially absorbs. These opposing requirements necessitate a very sharp drop in screening pigment absorption at about 600 nm, that is to say, a very sharp increase in transmission there. This is precisely what has been achieved in wild-type blowfly eyes (Fig. 1b).

Inevitably the rhodopsins do absorb some stray light even in wild type flies, and this results in noise. This artefact is of historic interest, as early measurements of the spectral sensitivity of fly eyes using the electroretinogram (ERG) yielded a distinct red peak. This was erroneously interpreted as evidence for the existence of a red receptor, but it was later correctly explained to be due to the experimental method (Goldsmith 1965).

Flies reveal a further subtlety. Their photoreceptors have a highly active pupil mechanism, mediated by yellow-coloured pigment granules located in the photoreceptor cell soma, which accumulate near the rhabdomere upon light adaptation. The functions of the pupil mechanism are manifold. Firstly, it controls the light flux travelling along the rhabdomere, thus expanding the intensity working range of the photoreceptor (Howard et al. 1987). Secondly, it narrows the angular sensitivity of the photoreceptors by absorbing preferentially from higher order waveguide modes (Smakman et al. 1984). Thirdly, the blue-green absorbing (Roebroek and Stavenga 1990a) and yellow transmitting (Fig. 1b) pupil favours photoreconversion of metarhodopsin, just like the red screening pigment (Stavenga 1980). The effective optical density of the pupil can be above 2 log units at the absorption peak wavelength, but in the yellow it is only 1 log unit (Roebroek and Stavenga 1990b). The pupil thus selectively suppresses light of the rhodopsin-converting wavelengths and transmits light of the metarhodopsin-reconverting wavelengths. The rhodopsin fraction of about 65%, resulting from white light entering on-axis with an open pupil, consequently increases to about 85% with a closed pupil (Stavenga 1980). The rhodopsin fraction can be increased further to about 95% or more with the assistance of red stray light, which has passed the red screening pigment. However, recalling the above considerations of noise created by the absorption of off-axis light, closure of the pupil causes a decrease in axial light flux and hence a decrease in the on-axis signal to off-axis noise ratio. Flies that have a highly active pupil, which severely reduces the axial light flux, therefore must install extra dense screening pigment.

The physiological conditions that shape the absorption spectra of screening pigment and pupillary pigments are distinctly different. The screening pigment is red to let stray light convert metarhodopsin, but not rhodopsin. The pupillary pigment can be yellow, because its main function is to gradually suppress the light flux in the rhabdomere, depending on the light intensity. A side effect of the mainly blue absorbing pupil is a shift of the photoreceptors' sensitivity spectrum with increasing light adaptation (Hardie 1979; Vogt et al. 1982).

The important conclusion is that screening pigment and pupillary pigment work in concert to photochemically restore the visual pigment, but that a good performance requires careful tuning of both their absorption spectra and optical densities. These brief considerations concern the so-called photosteady state. A more complete treatise of the economy of the visual and screening pigments should include the dynamics of the complete visual pigment cycle, i.e. it should encompass the enzymatic renewal pathway, the stages of phosphorylation and arrestin binding, and the dependence of the population of the different visual pigment states on the environmental light conditions.

Following this first insight of the tuning of screening pigment to visual pigment photochemistry, obtained from the blowfly (Stavenga et al. 1973), the same principle was argued to exist in male mayflies (Horridge 1976; Horridge and McLean 1978) and simuliid flies (Kirschfeld and Wenk 1976). The dorsal part of their eyes contain short-wavelength absorbing rhodopsins in combination with yellow pigments, similar to the well-studied dorsal eye of the owlfly *Ascalaphus*. The photoreceptors of this predatory insect contain a UV rhodopsin (R345), which is photo-interconvertible with a blue absorbing metarhodopsin (M475) (Hamdorf et al. 1973). The metarhodopsin is selectively reconverted by stray light which passes the yellow screening pigment (Langer 1975; Schneider et al. 1978).

A further excellent example of a tuned screening pigment is found in the dorsal eye of libellulid dragonflies; this eye region also plays a central role in predation. Here a blue absorbing rhodopsin (R420) is photoregenerated from a green absorbing metarhodopsin (M535) with the aid of an orange-brown screening pigment (Labhart and Nilsson 1995). As this pigment conspicuously colours the huge dorsal eye, its function

can be recognized by direct, visual inspection (Stavenga 1979, 1992). Furthermore, the dorsal eye of the honey bee drone, used for spotting queens, contains UV and blue receptors (Peitsch et al. 1992), together with reddish-brown screening pigment (Menzel et al. 1991; review by Stavenga 1992).

The striking difference between the absorption spectra of screening and pupillary pigment observed in the blowfly eye probably holds more generally. The fruitfly *Drosophila* has a bright red screening pigment paired with a greenish pupillary pigment, which absorbs mainly in the blue and UV (Franceschini and Kirschfeld 1976), and the dorsal photoreceptors of dragonflies contain yellow pupillary pigment granules (Warrant and Pinter 1990). These differences indicate that the precise shape of the pigment absorption spectra is determined by a number of species dependent requirements.

The screening pigments of most insect eyes are black to prevent activation of the photoreceptors by stray light

The photochemical pathway of vertebrate rhodopsin molecules ends in their thermal decay. They are subsequently reconstituted via a complicated enzymatic pathway. Invertebrate visual pigments are marked by the longevity of both the rhodopsin and the metarhodopsin state, but the metarhodopsins are nevertheless also degraded and renewed enzymatically (review by Schwemer 1989; Gärtner 2000). Why don't all insects apply the cheap, energy-saving tool of photochemical reconversion via stray light, as exploited by flies and a number of other insect species? The reason is that virtually all insects employ rhodopsins absorbing maximally in the green. It is a curious, but so far unexplained finding that rhodopsins with peak wavelength above ca. 500 nm are invariably photoconverted into a hypsochromically shifted, blue-absorbing metarhodopsin (Stavenga 1989, 1992). For those visual pigments a long-wavelength-leaky screening pigment would work detrimentally. The stray light then preferentially converts the rhodopsin, and the resulting noise is not compensated by metarhodopsin conversion. In other words, a spectral filter which selectively transmits long-wavelength light can only be used with visual pigments where the metarhodopsin is bathochromically shifted with respect to its rhodopsin. The screening pigment cells of most insect eyes are therefore packed with massive amounts of black pigment granules to protect the green rhodopsins. The strongly light absorbing pigment granules block out any light entering the eye from oblique, off-axial directions, thus ensuring that the photoreceptors capture light from a narrow spatial direction only, at all wavelengths. With little reflection from the screening pigments, the eyes have a black appearance, as for instance in most hymenopterans and papilionid butterflies.

In a photoreceptor which functions under natural, bright light conditions generally not more than about a third of the visual pigment molecules exist in the metarhodopsin state. This fraction is continually renewed, although with a limited speed. In the well-studied case of flies, where photoreconversion is a dominant factor, enzymatic visual pigment turnover is slow, taking several hours (Schwemer 1989). Butterflies, which have a high concentration of green rhodopsins, rapidly degrade their metarhodopsins, with a time-course of several minutes (Bernard 1983). Interestingly, the green-absorbing rhodopsin Rh6 of the fruitfly has a hypsochromic-shifted metarhodopsin, which rapidly decays thermally (Salcedo et al. 1999).

The bright colours of tabanid eyes may reflect a filter function of the multilayered facet lenses

The eye of the deerfly *Chrysops* (green-eye) *relictus* has a metallic gold-green colour, with dark red patches (Fig. 2a). The green colour is also seen in a cleaned, isolated cornea and therefore is intrinsic to the corneal facet lenses and not due to screening pigments (Bernard and Miller 1968). The phenomenon is caused by a multilayer, distally in the cornea, which consists of layers with alternately higher and lower refractive indices (Miller 1979). Microspectrophotometry shows that the reflection band is rather narrow (ca. 60 nm) and peaks at about 585 nm (Leertouwer and Stavenga 2000). The transmission is reduced in the reflection wavelength band, as is visible in a cleaned cornea from the darker facet lenses; where the facet lenses are non-reflecting they appear brighter in transmission (Stavenga 2001). The red colour of the dark patches is similar to that of blowfly eyes. The colour is indeed due to screening pigment, which is visible through transparent and low-reflecting facet lenses. The screening pigment is obscured in the green areas by the reflecting facets. The blowfly case suggests that the red screening pigment in the deer fly is also tuned to a blue-green absorbing rhodopsin in the R1–6 cells, with an orange absorbing metarhodopsin.

Reflecting corneal facet lenses are the hallmark of two dipteran families, the Tabanidae and the Dolichopodidae (Bernard and Miller 1968; Bernard 1971; Miller 1979). Transmission measurements of isolated corneas of various species showed that the transmission decrease can be 50% in *Chrysops*, but the changes in other species are often much less dramatic, or even very minor, resulting in low reflections (Lunau and Knüttel 1995). These minor reflections are still able to give a distinct eye colour, because the dark-brown screening pigments, which underly the corneal facet lenses, reflect even less.

Although the corneal reflections are very striking, their functional significance remains unknown (Friza 1929; Bernard 1971; Land 1972; Miller 1979; Lunau and Knüttel 1995). An attractive hypothesis, forcefully argued for the case of dolichopodids, suggests that the reduced transmission will modify the sensitivity spectrum of photoreceptors with suitable visual pigments, and will thus mediate improved colour contrast

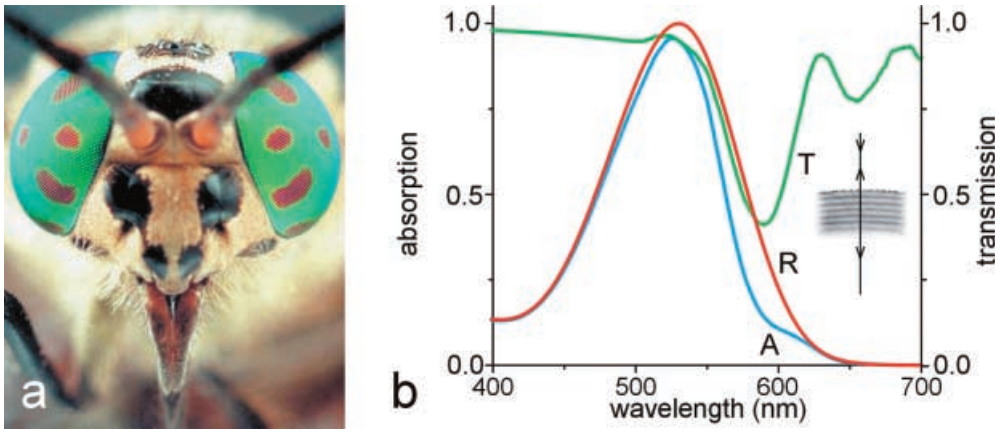

Fig. 2. The deer fly *Chrysops relictus* has gold-green eyes with dark-red patches (**a**). The green reflection is due to a multilayer in the facet lenses (**b** inset; Bernard and Miller 1968). The reflection spectrum peaks at about 585 nm. The complementary transmission spectrum (**b** green line, *T*) is assumed to have a minimal value of 0.4 at that wavelength (cf. (Lunau and Knüttel 1995). The corneal multilayer acts as a spectral filter on the underlying photoreceptors. It narrows the absorption spectrum (**b** blue line, *A*) of a photoreceptor having a rhodopsin peaking at 530 nm (**b** red line, *R*)

detection (Bernard 1971). Direct, physiological evidence is not available, but the concept can be illustrated with the simple model of Fig. 2b. The action of the corneal filter can be visualized by calculating the photoreceptor sensitivity spectrum, which is the product of the filter's transmission spectrum and the visual pigment's absorption spectrum [1]. The shape of the absorption spectrum can be deduced from visual pigment templates which have the value of the peak wavelength as the only parameter (Stavenga et al. 1993; Govardovskii et al. 2000). It is then easily seen that a 60-nm-wide spectral band filter peaking at 585 nm (Fig. 2b, green line) has negligible effects on visual pigments with absorption peak wavelength up to ca. 500 nm. As shown in Fig. 2b, when the filter acts on a rhodopsin with peak wavelength at 530 nm (Fig. 2b, dashed red line) the resulting sensitivity spectrum is substantially narrowed with respect to the unfiltered spectrum. In principle, therefore, photoreceptors with a rhodopsin peaking at about 530 nm, existing in ommatidia with and without the corneal filter, could collaborate in detecting colour contrast.

The candidates for the green-sensitive cells are the central photoreceptors R7 and R8 (Lunau and Knüttel 1995), assuming that the R1–6 photoreceptors indeed contain a blue-green rhodopsin (see above). Further physiological data are necessary to put these conjectures to the test. We may note that the central photoreceptors of flies mediate colour vision in blowflies (Fukushi 1989; Troje 1993). A specific colourful role has also been inferred for the central photoreceptors of dolichopodids, which have two types of spectrally conjugated corneal reflectors organized in extremely regular, interlaced patterns (Bernard 1971). In the dolichopodid *Condylostylus*, the reflection spectrum of one type is similar to that of *Chrysops*, and the other type is bathochromically shifted to a peak value of about 515 nm. When the two different filters act on photoreceptors equipped with the same rhodopsin peaking at ca. 550 nm, the resulting sensitivity spectra will be narrowed at the opposite flanks. Although direct evidence for this attractive hypothesis has yet to be obtained, the observation that the colour of the lenses in dolichopodids correlates with the microvillar direction of the underlying central photoreceptors indicates that the detection of colour and polarization differences is somehow combined (Bernard 1971; Trujillo-Cenóz and Bernard 1972; Land 1993).

It has been suggested that a possible function for the green corneal reflectors of *Chrysops* could be to reduce conversion of green rhodopsins by selectively suppressing long-wavelength light, thus favouring metarhodopsin reconversion (Lunau and Knüttel 1995). Although this might in principle work for the hypothesized green rhodopsin in an R7 or R8 cell, the net effect will be very minor. Moreover, it would work the opposite way in the presumed majority of R1–6 cells, making the hypothesis unlikely.

Even when the multilayers in the facet lenses modulate the transmission only slightly, as is the case for the horsefly *Haematopota pluvialis* (Lunau and Knüttel 1995), the eye colouring can be quite distinct (Fig. 3). The corneal reflectors therefore might have a display function, because the eye reflections will contribute to the insect's outward appearance. The female eye is marked by wavy bands of differently coloured facets extending throughout all eye regions (Fig. 3a). In stark contrast, the male eye is divided into two strongly different parts (Fig. 3b). The male ventral eye seems to

[1]Following general custom, I use the terms transmission, reflection and absorption in this paper in a rather loose way. Their correct meaning is the absolute value of the total amount of the incident light that is transmitted, reflected and absorbed, respectively. Transmittance, reflectance and absorptance are the fractions of the total amount of light that is transmitted, reflected, and absorbed, respectively. The absorbance is minus the decadic logarithm of the transmittance. The general loose usage of transmission, reflection and absorption spectrum is based on the general practice to normalize the spectra and that the normalized absorption, absorptance and absorbance spectra become identical for a thin layer of absorbing pigment.

Fig. 3a,b. The horse fly *Haematopota pluvialis* has eyes with wavy coloured patterns, due to reflecting multilayers in the corneal facet lenses. Whilst the patterns occur throughout the female eye (**a**), they exist only in the ventral part of the eyes of males (**b**). The white-grey colour of the large dorsal part of the male is due to screening pigments. The function of the coloured bands is unclear

be a copy of the corresponding eye part of the female, but the huge, dorsal eye is rather whitish. The dorsal eye is used to detect passing females, and presumably short wavelength receptors are prominent there, similar as in the whitish dorsal eye of mayflies (Horridge 1976). Given the minor ventral area of the male eye with respect to the large dorsal area, it seems somewhat unlikely that in this particular case the coloured ventral facet lenses perform an important display function. The striking patterning of tabanid eyes thus seems to hold a few secrets.

Coloured screening pigments can have a display function

With the function of the red screening pigments in the eyes of flies and dragonflies being understood, it may be thought that coloured screening pigments in insect eyes generally have some function in photoreceptor physiology. However, the colours usually have the main function of other body colours, namely to display a certain colour or pattern. In pierid butterflies, for example, whitish pigment overlays dense black screening pigment. The latter pigment achieves the usual goal of blocking out light, whereas the light-coloured pigment determines the eye's outward appearance. The distal pigment then does have an optical function, but only for display or blending the eye colour with the general body colour.

As an example, Fig. 4a shows the eye of the satyrine butterfly *Bicyclus safitza*. The eye is light-brown, with the dark and light bands typical for satyrines (Yagi and Koyama 1963). The whitish colour is due to distal pigment, covering black pigment. Again, the first pigment ensures that the eye colour is similar to that of the surrounding head tissue and the latter pigment has the usual function of blocking out stray light. The black pigment is clearly seen in the so-called principal pseudopupil, where the visual axes of the ommatidia are more or less aligned with the optical axis of the microscope

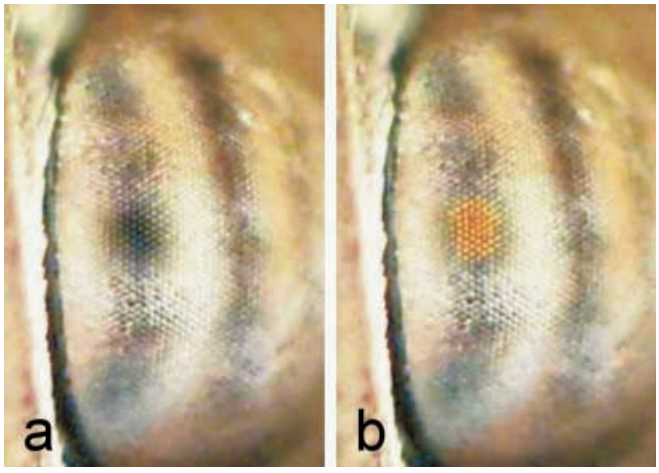

Fig. 4a,b. The eye of the satyrine butterfly *Bicyclus safitza* is coloured light brown with dark dorso-ventral bands. The light brown colour is due to light-reflecting screening pigment located distally in the screening pigment cells. The dark colour is due to black screening pigment situated more proximally, around the cones. The central dark spot is the principal pseudopupil (**a**). The ommatidia there are more or less aligned with the viewing microscope. The dark pseudopupil is especially well seen with oblique illumination, because of the little scattering by the dark pigment (Stavenga 1979). It also stands out with epi-illumination after sufficient light adaptation (achieved after a few seconds; the situation photographed in **a**. The principal pseudopupil features a marked eye shine in the dark-adapted state (**b**), due to light reflected at the tapetum, positioned proximally to the rhabdom. The number of shining ommatidia depends on the aperture of the microscope objective (here an Olympus ×5, NA 0.10). The pseudopupil (of **a** and **b**) is confined to the dorsal eye part where the eye shine is homogeneous (Stavenga 2002)

(for more detailed discussion and examples, see Stavenga 1979, 1989).

The orange eye shine of many butterfly eyes is mainly due to visual pigment absorption

Butterflies, except for the family Papilionidae, have in each ommatidium a reflecting tapetum proximal to the rhabdom. Incident light, which enters the eye and is guided through the rhabdom without being absorbed, is reflected by the tapetum. When it travels another time through the rhabdom without being absorbed and leaves the eye again, it can be observed in a dark-adapted eye, with an epi-illumination microscope, as the so-called eye shine (Fig. 4b). Illumination rapidly extinguishes the eye shine due to activation of a pupil mechanism, similar to that existing in fly photoreceptors (Stavenga et al. 1977; Stavenga 1979), leaving a black pseudopupil (Fig. 4a).

A survey of several butterfly species reveals that the eye shine can strongly vary over the eye (Stavenga et al. 2001). The distribution of the eye shine gradients can be readily studied with a newly developed setup (Stavenga 2002). The heart of the setup is a large-aperture microscope objective with a long-working distance. In addition, two diaphragms in the illumination and image

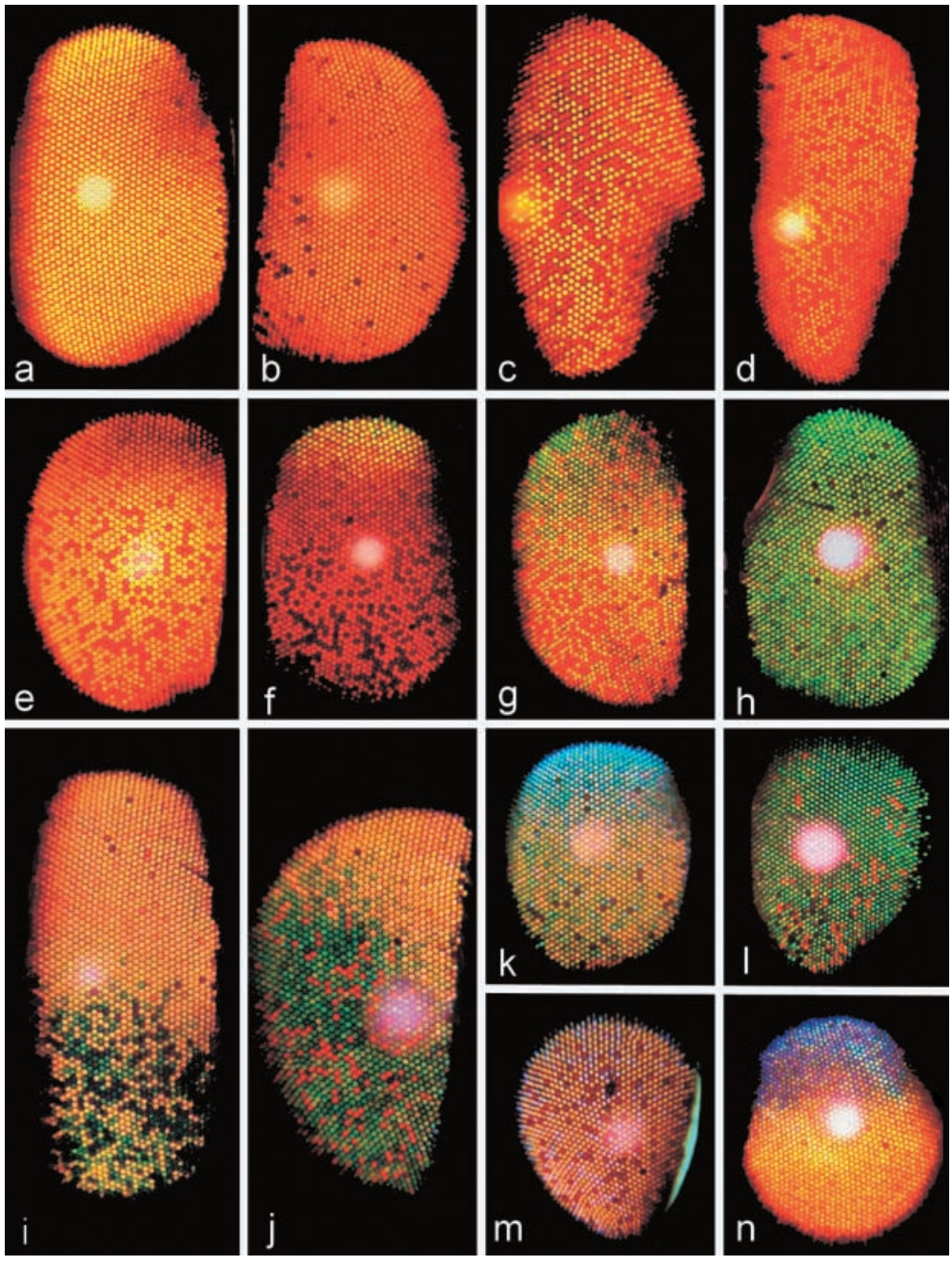

Fig. 5. Butterfly eye shine photographed with a large aperture epi-illumination setup: **a** comma, *Polygonia c-album*; **b** peacock, *Inachis io*; **c** glider, *Cymothoe herminae*; **d** forest pearl charaxes, *Charaxes fulvescens*; **e** lycaenid, *Narathura japonica*; **f** small white, *Pieris rapae*; **g** speckled wood, *Pararge aegeria*; **h** ringlet, *Aphantopus hyperantus* ; **i** variable eggfly, *Hypolimnas anthedon*; **j** blue mother-of-pearl, *Salamis temora*; **k** lycaenid, *Pseudozizeeria maha argia* ♂; **l** satyrine, *Ypthima argus* ♂; **m** lycaenid, *Everes argiades hellotia* ♀; **n** map butterfly, *Araschnia levana*. The eye shine pattern can be virtually homogeneous yellow/orange (**a**, **b**), or a random mixture of yellow and orange (**c**, **d**) in all eye regions. More often a distinct dorsal region exists, which is rather small (**e**–**g**, **k**, **n**), or large (**i**, **j**, **l**), with a more or less homogeneously coloured eye shine. A rich mixture of differently coloured ommatidia usually marks the ventral area, often with a distinct red component (**e**, **g**, **j**–**m**). The dark ommatidia in **f** reflect well in the deep-red (Qiu et al. 2002; Stavenga 2002)

beam, respectively, are placed confocal with the deep pseudopupil (DPP) and adjusted to the DPP size (Franceschini and Kirschfeld 1971). Because the visual axes of the ommatidia intersect in the DPP, the eye shine is then effectively separated from the reflections emerging from other eye structures, e.g. the corneal facet lenses and the reflecting pigments in the pigment cells. Some remnant reflections due to light reflected at the objective lens surfaces cause the 'hot spot' seen in Fig. 5a–n.

Figure 5 illustrates that each butterfly species has its characteristic eye shine pattern (Bernard and Miller 1970; Miller 1979; Stavenga et al. 2001). Occasionally the colours emerging from the individual facet lenses are very similar, yielding a homogeneous eye shine pattern (Fig. 5a, b). More frequently, a homogeneous colour occurs only in a more or less limited dorsal eye part, whilst the remaining, ventral part of the eye is very heterogeneous (Fig. 5e, f, i, j, k, m). The heterogeneity sometimes occurs throughout all eye regions, with everywhere the same colour combinations (Fig. 5c, d), or, with different combinations (Fig. 5g, k). In some cases the dorsal and ventral areas are both more or less homogeneous, although quite differently coloured (Fig. 5m). The usual eye shine colours are in the long wavelength range (Fig. 5a–g), but occasionally a dominant green is seen (Fig. 5h, l). A blue or even violet eye shine can be seen dorsally (Fig. 5k, n).

The common yellow-red eye shine can be understood with a simple model of a butterfly ommatidium, which is based on our present knowledge of the small white, *Pieris rapae* (Qiu et al. 2002), and the Japanese yellow swallowtail, *Papilio xuthus* (Arikawa and Stavenga 1997; Arikawa et al. 1999a). These butterfly species have a tiered rhabdom. The dominant layer, taking up about the distal two-thirds of the rhabdom, is composed of the rhabdomeres of the four distal cells, R1–4. The proximal one third consists almost completely of R5–8 rhabdomeres, and a short basal part is due to an R9 rhabdomere. Extensive physiological and histological studies on *P. xuthus* have shown that the eyes have three classes of ommatidia (review by Arikawa 1999).

Averaging over the different classes yields an ommatidium where the distal part has about equal amounts of an ultraviolet (UV), blue (B), and green (G) absorbing rhodopsin, and the proximal one third of the rhabdom has exclusively green rhodopsin (see Fig. 6a, inset). Taking a rhabdom length of 400 μm (Qiu et al. 2002), known visual pigment templates (Stavenga et al. 1993), rhodopsins with peak wavelengths 360 nm (UV), 450 nm (B) and 540 nm (G), respectively (Fig. 6a), and a visual pigment extinction coefficient of 0.005 μm^{-1} (Warrant and Nilsson 1998), the resulting decrease in light flux in the rhabdom can be calculated (Fig. 6b). The dotted line D in Fig. 6b represents the transmission decrease due to only the distal layer, and the dashed line P in Fig. 6b gives the decrease due to the distal layer together with the proximal layer. After reflection on the tapetal mirror and a double pass back through the rhabdom, the eye shine reflection is then given by line E (Fig. 6b); it is assumed here that the tapetum reflects with a wavelength-independent efficiency of 0.9. Clearly, the rhabdom-tapetum combination works as a long-pass spectral filter, strongly reducing the light intensity at all but the long wavelengths. Whether the colour of the remaining light is orange or red will largely depend on the peak wavelength of the green rhodopsin.

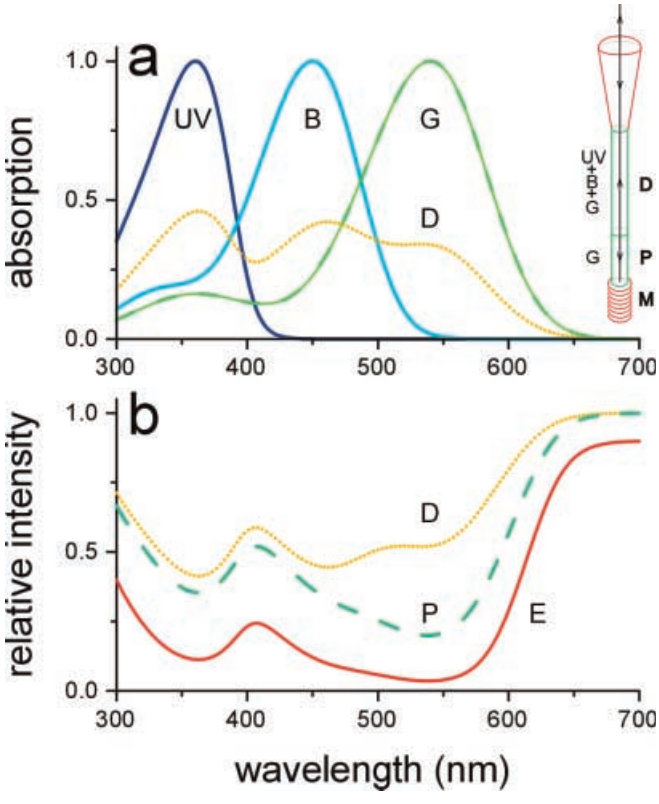

Fig. 6a,b. Modelling butterfly eye shine. The distal two thirds of a 400 μm rhabdom (**a** inset, *D*) is assumed to be filled with equal amounts of an ultraviolet- (*UV*), blue- (*B*) and green-absorbing rhodopsin (*G*), with peak wavelengths of 360 nm, 450 nm and 540 nm, respectively. The proximal one-third of the rhabdom contains exclusively the green rhodopsin (**a** *dashed line*, coincident with *G*). The *dotted line* (**a** *D*) indicates the resulting averaged absorption spectrum of the distal rhabdom relative to the absorption in the proximal rhabdom. The reduction in light flux due to the first pass through the distal rhabdom (**b** *D*) as well as through the proximal part (**b** *P*) is substantial throughout the main part of the spectrum, but it is minor in the long-wavelength range. Assuming a 90% reflection by the tapetal mirror (**a** inset, *M*) at all wavelengths, a double pass through the rhabdom results in an eye shine, which is dominated by orange-red wavelengths (**b** *E*). The absorption coefficient of rhabdom tissue containing only one rhodopsin is assumed to be 0.005 μm^{-1}. Waveguide effects have been neglected to keep the model simple

Of course, the assumption of a spectrally flat tapetal reflector is an oversimplification. Measurements show that the tapeta have broad reflection spectra (Ribi 1979a; Bernard and Remington 1991), but the spectra always cut off at some wavelength. The cut-off value distinctly depends on the specific type of tapetum (Bernard and Remington 1991; Stavenga 2002). The eye shine in the dorsal area of many species can be distinctly yellow (small white, Fig. 5f), green (speckled wood, Fig. 5g) or even blue-violet (map butterfly, Fig. 5n), demonstrating that the tapetal mirrors then are effective in a limited wavelength range only. The reflection properties of the tapetum thus will distinctly modify the eye shine spectrum (E) derived in Fig. 6b.

When the eye shine is green (Fig. 5h, l), the cut-off wavelength is well below 600 nm. Figure 6 shows that in this case the intensity of the reflected light is much smaller than that with a tapetum extending its reflection spectrum into the red. The resulting exposure time for photographing the eye shine thus goes up considerably, causing a bright 'hot spot' (Fig. 5h, l).

The obvious question then is whether the ubiquitous heterogeneous colours seen in Fig. 5 are fully determined by the differences in the tapetal reflection spectra. Microspectrophotometrical measurements show that the situation is often more complicated and that the participation of additional pigments must be assumed (Stavenga 2002).

Butterfly ommatidia reflecting in the red combine a red reflecting tapetum with a screening pigment absorbing at short wavelengths

Anatomical work on the small white, *Pieris rapae*, has revealed that this butterfly has three anatomically dis-

tinct classes of ommatidia in the major, fronto-ventral part of the eye; in two classes, four clusters of the same light-red coloured pigment exist around the rhabdom, and in the other class four clusters of deep-red coloured pigment are found (Qiu et al. 2002). The three classes of ommatidia intermingle in a random fashion. The pigment clusters act as spectral filters which strongly affect the eye shine, yielding red and dark-red facets, respectively (Fig. 5f; Ribi 1979b; Stavenga 2002).

Figure 5 shows that red-reflecting ommatidia occur abundantly in the eyes of many species and in different families (see also Stavenga et al. 2001). The red reflections can now be interpreted by extrapolating the firm evidence for photoreceptor screening pigments present near the rhabdom boundary. Photostable pigments absorbing in the major part of the visible range and transmittant in the red have been demonstrated to exist in photoreceptors of papilionids (Arikawa and Stavenga 1997; Arikawa et al. 1999b), pierids (Ribi 1978a, 1979b; Qiu et al. 2002) and also in hymenopterans, *Sphex* (Ribi 1978b). In these cases the red filter pigments function to create red receptors: in papilionids (Arikawa and Uchiyama 1996; Arikawa et al. 1999b), pierids (Ribi 1978a; Steiner et al. 1987; Scherer and Kolb 1987), and *Sphex*: (Ribi 1978c). The ommatidia of other butterfly species reflecting in the red can hence be hypothesized to harbour red transmittant filters as well, resulting in photoreceptors with sensitivity spectra peaking in the red (Stavenga 2002). The present conjecture that red receptors are widely present in butterfly visual systems will require extensive anatomical and electrophysiological work to be validated. The eye shine patterns show that the red reflecting ommatidia are randomly distributed between ommatidia with different spectral characteristics (Fig. 5). Heterogeneity appears to be a common feature of insect eyes (Arikawa and Stavenga 1997; Stavenga et al. 2001).

The foregoing shows that the main factors determining the eye shine colours are the absorption spectra of the visual pigments existing in the rhabdom, the reflection spectra of the tapeta, and, in many but certainly not all cases, the absorption spectra of pigments concentrated in granules assembled near the rhabdom (Miller 1979; Stavenga 1979, 2002).

Colours seen by insect eyes

Colour vision is presumably widespread among insects, as multiple rhodopsin types have been identified in most species investigated. Extensive knowledge exists of the colour vision system of the honeybee, where three photoreceptor classes (UV, B and G) form the standard set of photoreceptors underlying colour vision (Menzel and Backhaus 1989). Convincing evidence for colour vision in other insects has been obtained only recently. The photoreceptor cells mediating colour vision in the blowfly *Lucilia*, the central photoreceptors R7 and R8 (Fukushi 1989; Troje 1993), exist in two pairs with different visual pigments. The two pairs are randomly distributed in the retina, i.e. in a heterogeneous pattern (Salcedo et al. 1999).

For papilionid butterflies, which have a specifically well-developed colour vision system (Kinoshita et al. 1999; Kinoshita and Arikawa 2000; Kelber and Pfaff 1999; Kelber et al. 2001), the responsible photoreceptors have not yet been identified. At least the photoreptors in the distal part of the retina must participate, as it is here that the UV, B and G receptors are located (Arikawa et al. 1999a; Kitamoto et al. 2000). However, the proximal photoreceptors must also be involved, because the yellow and red photoreceptor screening pigments modify the sensitivity spectra of the cells in the proximal part of the rhabdom (Arikawa et al. 1999a), and red receptors dominate the proximal tier of the retina in *Papilio* (Arikawa and Uchiyama 1996). It is highly likely that the short basal receptors play a role in the discrimination of red light. In ommatidia with red-filtering screening pigment in the distal photoreceptors, red sensitivity of the basal receptors is relatively enhanced. This red sensitivity will be further enhanced when a tapetum exists which reflects well in the long-wavelength range. Outstanding examples are the eyes of Pieridae, where the two types of red filters (Qiu et al. 2002) are combined with two tuned types of tapeta (Qiu et al. 2002; Stavenga 2002). Ample behavioural evidence indicates that colour discrimination in the red is well-developed in *Pieris* (Kolb and Scherer 1982; Scherer and Kolb 1987) and other butterflies (Ilse 1937; Kinoshita et al. 1999; Kelber 1999a).

Inserting filters to improve spectral discrimination seems to be a standard technique in insect vision. For instance, *Papilio xuthus* also uses a far-UV filter to sharpen a UV-rhodopsin-containing photoreceptor into a violet receptor (Arikawa et al. 1999a). However, before we fully understand how and why the different methods are applied and combined, extensive experimental work will be necessary.

In males of several insect species, as well as in predatory insects, dorsal eye areas are specialized for spatial contrast vision (e.g. Fig. 3). As discussed above, in these cases only one or two visual pigments are expressed. Colour vision thus may have given way to spatial image processing in these eye regions. The ventral eye areas presumably do mediate colour vision, as for instance in the ventral area of the honeybee drone which has the normal three (UV, B and G) bee photoreceptors (Peitsch et al. 1992). We may speculate that the dorsal eye area of male tabanids is devoted to monochromatic contrast vision with high spatial acuity, and that the ventral area processes colour vision only, like in the drone. Although a strong functional dichotomy between dorsal and ventral eye areas may not be the rule for all insects, a division of labour between different eye areas seems to be frequently present. In butterflies, the colouring of the dorsal eye shine patterns is often rather homogeneous, opposed to the rich colouring in the ventral area. This seems sensible, as it infers that an elaborate spectral

processing system is installed in the downward looking eye area, to detect food sources via colour contrast. Careful tuning to the spectral characteristics of the objects to be detected may be expected.

In addition to spatial and colour vision, many insects, notably bees, utilize polarization vision (Rossel 1989). Polarization vision in flies occurs in a narrow dorsal rim, via UV photoreceptors (Hardie 1986). Crickets employ a prominent dorsal area, recognizable by smooth facet lenses and ommatidia void of screening pigment (Burghause 1979; Labhart et al. 1984; Stavenga 1989) with exclusively blue-sensitive photoreceptors (Burghause 1979; Labhart et al. 1984; Stavenga 1989). Intriguingly, papilionid butterflies seem to deliberately confuse colour and polarization information for specifically enhancing the visibility of certain objects, like fresh leaves for oviposition (Kelber 1999b).

The enormous variation in the organization of insect eyes suggests that the different visual modalities, i.e. spatial, colour and polarization vision, have different weighting factors for biological fitness. Presumably they will depend on the characteristics of the visual space to be sampled. When polarization detection is at a premium, as in bees, ants, and crickets, it can occupy a large dorsal eye area. When detection of prey or a mate is the goal, as in dragonflies and drone bees (and many other male insects), a major dorsal eye region can be similarly dedicated. And when the strategy is rather for catching prey in forward directions, as in robberflies and mantids, a large frontal eye area is utilised (Stavenga 1979). Usually, eye regions with different functions will not be clearly separated, and overlapping gradients will exist.

From the heterogeneous eye shine patterns it seems that in some butterfly species colour vision elaborately occurs in all eye regions, but in other butterfly species a high degree of colour processing seems to be restricted to the ventral area. Physiological and behavioural measurements need to underscore these speculations. To resolve how tuned spectral sensitivities are used in the processing of colour is a huge task. A strong leitmotiv in these studies is the tremendous heterogeneity of butterfly eyes, so beautifully visible in their eye shine colours.

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