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Tuning of Photoreceptor Spectral Sensitivities by Red and Yellow Pigments in the Butterfly *Papilio xuthus*

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ABSTRACT—The compound eye of the Japanese yellow swallowtail butterfly, *Papilio xuthus*, consists of different types of ommatidia characterized by the pigmentation around the rhabdom. About 75% of the ommatidia harbor red pigment, whereas the other 25% contain yellow pigment. We find that the pigments function as spectral filters for the proximal photoreceptor cells. Intracellular recordings of the proximal cells yielded spectral sensitivities peaking in the red (λ_{max} = 600 nm) and in the green (λ_{max} = 520 nm), respectively. Staining of the recorded cells and subsequent histology demonstrated that the red receptors contain red pigment and that the green receptors contain yellow pigment. The sensitivity spectrum of the red receptors was considerably narrower compared to the absorption spectrum of a visual pigment peaking at 600 nm. The sensitivity spectrum can be calculated with an optical model for the butterfly rhabdom by incorporating measured absorbance spectra of the red pigments, yielding that the cell contains a visual pigment peaking at about 575 nm. The model also indicated that the spectral sensitivity of the green receptors is produced by the combination of the yellow lateral filter and a visual pigment peaking at 515 nm.

INTRODUCTION

Photoreceptors are sensitive to light because their visual pigment molecules are converted upon absorption of photons. The absorption spectrum of the visual pigment therefore determines the photoreceptor's spectral sensitivity. However, frequently the spectral sensitivity is modified by optical effects, e.g., due to self screening, filtering by photostable pigments, and/or by the waveguide properties of the organelle containing the visual pigment (Stavenga, 1992).

A comparison of the spectral sensitivity of the red receptor of *Papilio xuthus* (Arikawa *et al*., 1987) with absorbance spectra predicted for visual pigments (Stavenga *et al*., 1993) revealed that the spectral sensitivity is considerably narrower than expected. A red receptor with a narrow spectral profile was reported previously for the cabbage butterfly, *Pieris rapae* (Shimohigashi and Tominaga, 1991). The spectrum has been interpreted to result from an orange absorbing visual pigment, filtered by a red pigment shown to exist by anatomy and in optical experiments (Ribi, 1979). Anatomical sections of the *Pieris* retina reveal that the red filter is made up of pigment granules, clustered adjacent to the rhabdom in four proximal photoreceptor cells (Ribi, 1978a). Clearly, the pigment is well-suited to act as a short-wavelength absorbing filter. Because retinal sections of the orchard swallowtail, *Papilio aegeus*, feature a very similar arrangement of four pigment clusters near the rhabdom (Ribi, 1987), it was obvious to conjecture that red pigment near the rhabdom also causes the narrow-band red spectral sensitivity profile in *Papilio*.

To understand the spectral characteristics of the *Papilio* photoreceptors into more quantitative detail, we reinvestigated the anatomical organization of the ommatidia with special focus at the pigment properties. Surprisingly, we thus found that not all ommatidia have the red pigment, i.e., one fourth of the ommatidia appears to have a yellow pigment instead (Arikawa and Stavenga, 1997). This immediately suggested an explanation of the ommatidial heterogeneity in *Papilio* reported by Bandai *et al*. (1992), who indicated that a certain anatomical cell type can have a variety of spectral sensitivities. We hence investigated the consequences of the differences in ommatidial pigmentation by further electrophysiological measurements of the spectral sensitivities of photoreceptors and by developing a simple optical model for the *Papilio* rhabdom, which functions as an optical waveguide (Nilsson *et al*., 1988). The waveguide optical model predicts that the red pigment acts as an optical filter for an orange absorbing visual pigment, resulting in a strongly shifted and narrowed sensitivity spectrum of the red receptors. The effect of the yellow pigment on the sensitivity spectrum of the green receptors ap-

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pears to be less striking.

MATERIALS AND METHODS

Animals

We used spring form males of the Japanese yellow swallowtail butterfly, *Papilio xuthus*, within 3 days after emergence. The butterflies were reared on fresh citrus leaves at 25°C under a light regime of 8 hr light : 16 hr dark. The pupae were stored at 4°C for at least 3 months, and then allowed to emerge at 25°C.

Histology

For light microscopical histology, the compound eyes were isolated and immersed in 4% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 (CB) at room temperature for 30 min. The tissues were then dehydrated with an acetone series and embedded in Spurr's resin. 10-14 μm thick sections were cut with a microtome and then observed under a Nomarski interference microscope (BX-60, Olympus) without staining. For electron microscopy, the isolated eyes were prefixed, in 2% glutaraldehyde and 2% paraformaldehyde in CB at room temperature for 2 hr, and subsequently postfixed, in 2% OsO_4 in CB at room temperature for 2 hr. The tissues were then dehydrated in an acetone series and embedded in Epon. Ultrathin sections, cut with a diamond knife, were double stained, with uranyl acetate and lead citrate, and subsequently observed with an electron microscope (JEM 1200EX, JEOL).

Microspectrophotometry

The compound eyes were isolated from the head, put in a plastic well filled with OCT compound, and rapidly frozen by immersing it in cold (ca. –50°C) n-hexane. 6-10 μm thick sections were mounted on a poly L-lysine-coated slide, and kept at 4°C covered with aluminium foil until use.

The absorbance spectra of the red and yellow pigments were measured from pigment clusters in the frozen sections. Before the actual measurement, we first illuminated the specimen with dim light back through the fiber optics attached to the microscope. This allowed us to accurately identify the measuring site as a light spot. The light spot, ca 1.0 μm in diameter, was then carefully positioned at a strip of pigmentation in an obliquely-cut retinal region that did not include a part of the rhabdom, so to avoid any possible contamination by the visual pigment absorption. Moreover, the area around the light spot was covered with an adjustable X-Y diaphragm system set between the tip of the fiber optics and the microscope optics to minimize the effect of stray light. The fiber optics was then removed from the light source and connected to a photodiode array (USP-410, Unisoku) equipped with an image intensifier (V1366U, Hamamatsu photonics) and a monochromator to measure the light passed through the diaphragm.

Electrophysiology

Electrophysiological methods were as described before (Bandai *et al.*, 1992). Briefly, a butterfly was mounted in a Faraday cage, and a glass micropipette, filled with 5% lucifer yellow CH in 0.2M LiCl, was inserted into the retina through a hole made in the dorsal cornea. After impalement of a single photoreceptor, first its spectral sensitivity was determined. The stimulus was a point source, adjusted to the optical axis of the cell, delivering light pulses that were monochromatic (range 290 to 700 nm) and equi-quantal (maximal intensity 5.0 $\times 10^{11}$ quanta cm^{-2} s^{-1} at the corneal surface). Subsequently the polarization sensitivity of the photoreceptor was measured. The recording was concluded to be from a proximal photoreceptor when i) the peak of the spectral sensitivity (λ_{max}) was in the green or red wavelength region, and ii) the peak of the e-vector orientation of the polarization sensitivity was either at about 35° or 145° with respect to the sagittal plane (Arikawa and Uchiyama, 1996). If that was the case, we injected lucifer yellow into the cell by applying 2 to 10 nA of hyperpolarizing DC current for several minutes. Finally, the eye was fixed and embedded in Spurr's resin for histology.

Structure of the *Papilio* ommatidium

Figure 1 schematically represents the structure of the ommatidium of *Papilio xuthus*. The rhabdom is three tiered with the total length of about 500 μm, and is made up of the rhabdomeres of 9 photoreceptor cells. The distal tier of the rhabdom (*D*, 260 μm) consists of the rhabdomeres of cells R1-4. Going from distal to proximal, there is a transitional zone (*T*, 260-330 μm), where the rhabdomeres of R1-4 gradually vanish and those of cells R5-8 emerge. The proximal tier (*P*, 330-470 μm) of the rhabdom consists entirely of the rhabdomeres of cells R5-8. Most proximally, the basal photoreceptor R9 contributes the microvilli to the basal tier of the rhabdom (*B*, 470-500 μm). The photoreceptors R3-8 in an ommatidium have yellow or red screening pigment clusters adjacent to the rhabdom (Arikawa and Stavenga, 1997). A UV absorbing (non-visual) pigment exists distally

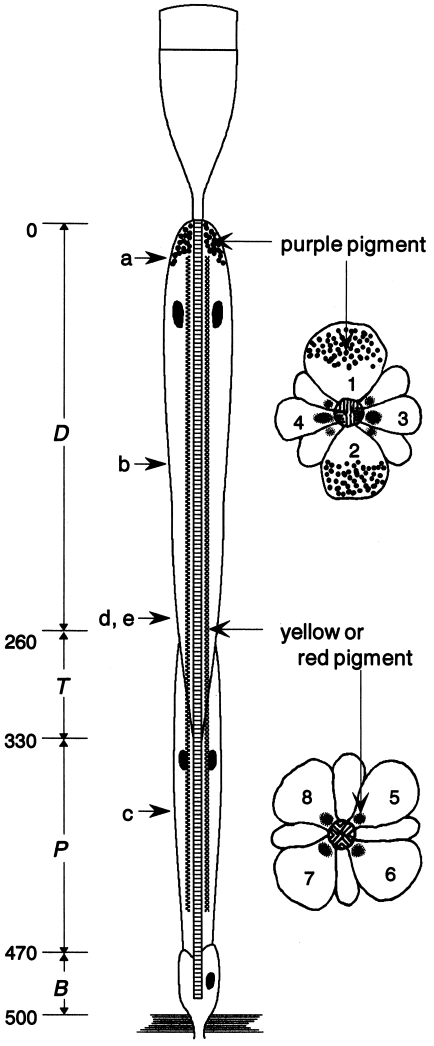

Fig. 1. Diagrammatical sketch of a *Papilio* ommatidium. The ommatidium of *Papilio* contains 9 photoreceptor cells (1-9), and is three tiered (*D*, distal tier; *P*, proximal tier; *B*, basal tier) with a transitional zone (*T*) between *D* and *P*. Numbers on the left side indicate the distance from the rhabdom tip in micrometer for an ommatidium in the lateral looking eye region. Letters also on the left side (a-e) indicate the depths where micrographs Fig 3a-e were obtained.

in a restricted number of ommatidia (Arikawa and Stavenga, 1997). Its characteristics and optical effects have been described elsewhere (Arikawa *et al.*, 1999). We do not explicitly treat the UV-absorbing pigment here, as we wish to concentrate on the spectral changes occurring in the long wavelength range. Yet, we have incorporated the pigment in the calculations with the model described below.

Modeling

We have calculated the absorption of light in the individual proximal photoreceptors (R5-8) with an optical waveguide model for the rhabdom (Fig. 2). The change in light flux along the rhabdom is described by (cf. Snyder *et al.* 1973):

$$dI(z, \lambda)/dz = -\{\eta(\lambda) \Sigma f_j(z)\, \kappa_j(z)\, \alpha_j(\lambda) + [1-\eta(\lambda)]\kappa_s(z)\, \alpha_s(\lambda)\} I(z, \lambda)$$

where $I(z, \lambda)$ is the light flux at a distance z from the tip of the rhabdom; $\eta(\lambda)$ is the fraction of the light flux propagated within the rhabdom boundary; λ is the light wavelength; f_j is the fraction of the rhabdomere cross-section taken up by photoreceptor R_j (j = 1-9); κ_j is the peak absorbance coefficient of the rhabdomeric tissue of photoreceptor Rj that contains the visual pigment j; κ_s is the peak absorbance coefficient of the cell body area around the rhabdom containing the (red or yellow coloured) screening pigment (Fig. 2); and α_j and α_s are the normalized spectral absorbance coefficients of the visual and screening pigments, respectively. The light absorbed by the visual pigment in each photoreceptor, integrated over the photoreceptor's length yields its absorption spectrum. Normalization then yields the spectral sensitivity.

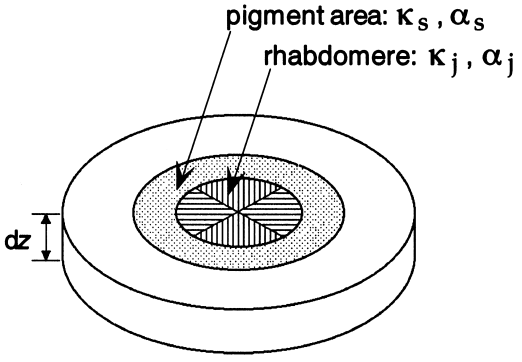

Fig. 2. A thin slice of the idealized ommatidium for model calculations. For details, see text.

We have modeled the rhabdom as a circular-cylinder with diameter 2.6 μm and length 500 μm (Fig. 1). The light outside the waveguide can interact with the red or yellow screening pigment in the photoreceptors near the rhabdom boundary. The screening pigment not only exists in the proximal cells R5-8, but also in the distal cells R3 and R4 (see Results). We assume that the density of the screening pigment in the photoreceptors is constant along the whole rhabdom length.

In the model, the rhabdomeres of R1-4 stretch from 0 to 300 μm and contain various combinations of UV, blue, and green visual pigments (Bandai *et al.*, 1992), with or without the UV absorbing screening pigment (Arikawa *et al.*, 1999). The proximal photoreceptors R5-8 extend from 300 to 470 μm and contain an orange absorbing or a green absorbing visual pigment (Arikawa and Uchiyama, 1996). The visual pigment spectra are described by template formulae (Stavenga *et al.*, 1993). In the calculations we took $\kappa_j = 0.005\ \mu m^{-1}$ for j = 1-9 and $\kappa_s = 0.05\ \mu m^{-1}$ (see below). The lowest waveguide mode was assumed to be the dominant component of the light flux propagated in the rhabdom. The fraction of light within the rhabdom therefore was approximated by: $\eta(V) = a - b\exp(-c\,V)$, with $a = 0.96$, $b = 2.82$, $c = 1.27$ and $V = \pi d\,(n_1^2 - n_2^2)^{1/2}/\lambda$, with $d = 2.6$ μm, $n_1 = 1.36$ and $n_2 = 1.34$ (Smakman and Stavenga, 1986; see further Arikawa *et al.*, 1999).

RESULTS

Photoreceptor cell pigmentation

We have investigated the anatomy of the retina of *Papilio* by light microscopy in unstained plastic sections. The distal photoreceptors R1 and R2 in all ommatidia contain dark purple pigment in the distal tip of the cell, close to the crystalline cone (Fig. 3a). The distal photoreceptors R3 and R4 contain pigment clusters close to the rhabdom (Fig. 3b). The same pigment clustering is found in the photoreceptors contributing to the rhabdom in the proximal layer, R5-8. In transversal sections, the pigment clusters are clearly seen as four densely colored spots around the rhabdom, in a more or less quadrangular pattern (Fig. 3c). The pigment is not fully concentrated. A small fraction is slightly spread out over the cell soma. We observed the position of these pigments in both light and dark adapted eyes, but could not see any clear indication of pigment migration. The soma of the basal photoreceptors, R9, has no evident pigmentation.

Interestingly, the color of the pigment in all R3-8 cells in an ommatidium is identical, but, surprisingly, the color varies among the ommatidia. Most ommatidia contain red pigment, but others harbor yellow pigment. We hereafter refer to the ommatidia containing red pigment as the R-ommatidia, and, similarly, we call the ommatidia with yellow pigment the Y-ommatidia. The relative occurrence of the type of color in the lateral-looking eye region is about 3:1, i.e., of 653 ommatidia, 487 contained red pigment and 166 yellow.

Electron microscopy demonstrated that the pigmented spots are clusters of irregularly shaped pigment granules (Fig. 3d, e). The pigment granules are clustered in the cell body area at 1.0 - 1.5 μm from the boundary of the rhabdom. The electron density of the red pigment granules is higher than that of the yellow pigment granules. Furthermore, the rhabdomeric microvilli of photoreceptors R1-4 in R-ommatidia appear to curve (Fig. 3d), whereas the microvilli of R5-8 in R-ommatidia, as well as those of all R1-8 in Y-ommatidia, are straight (Fig. 3e).

The pigment clusters exist near the boundary of the rhabdom, which functions as an optical waveguide due to its slenderness (Stavenga, 1979; Nilsson *et al.*, 1988). The pigments therefore will affect the light propagation in the rhabdom, i.e. they function as an optical filter (see Arikawa and Stavenga, 1997). The pigment clusters in the R3-4 cells are optimally positioned to act as an absorption filter for the proximal cells. The pigment in the proximal cells proper will further enhance the filtering. In order to estimate the filtering effect on the sensitivity spectra of the photoreceptors, we measured the transmittance spectra of the red and yellow pigment clusters (Fig. 4). Clearly, the red and yellow pigments are virtually transparent above 580 nm and 500 nm, respectively. We approximated smooth lines to the red and yellow spectra, respectively. The absorbance spectra of the two pigment types thus obtained

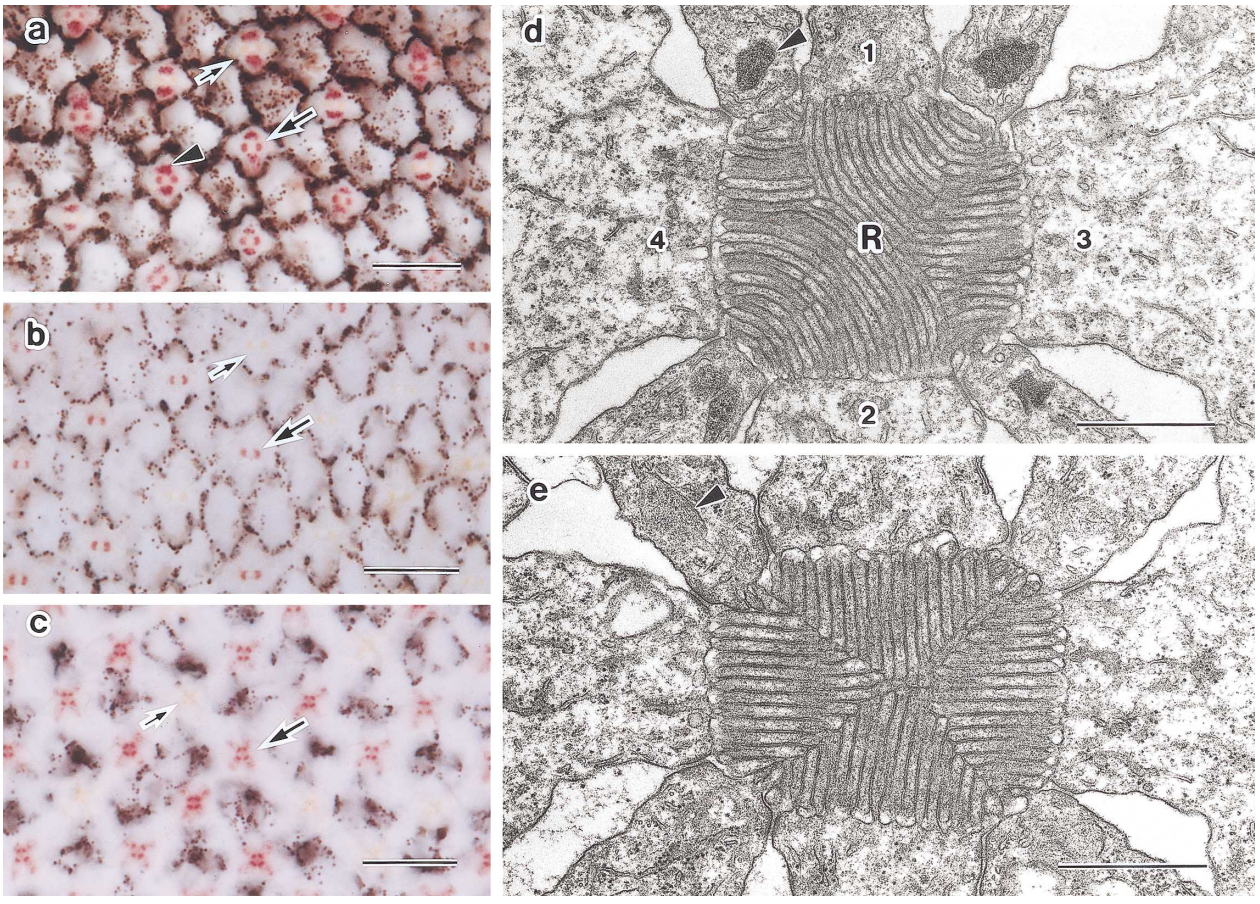

Fig. 3. Light and electron micrographs of transverse sections of the retina of *Papilio*. **a** Immediately proximal to the crystalline cone. Two of the distal photoreceptors, R1 and R2, in all ommatidia contain purple pigment (arrowhead). Large and small arrows indicate red pigmented (R-) and yellow pigmented (Y-) ommatidia, respectively. **b** Middle of the distal tier. The other two distal photoreceptors, R3 and R4, contain clusters of red (large arrow) or yellow (small arrow) screening pigments. **c** Proximal tier. All four proximal photoreceptors, R5-8, contain pigment clusters. The color of the pigment is the same as those in R3,4 in the same ommatidium. **d** R-ommatidium, cut through the region slightly distal to the transitional zone (see Fig. 2). The pigment clusters appear electron-dense (arrowhead). The rhabdomeral microvilli of R1-4 are curved, whereas those of R5-9 are straight (not shown). **e** Y-ommatidium. The pigment clusters appear electron-lucent (arrowhead). The rhabdomeral microvilli of R1-4, as well as R5-9 (not shown) are straight. 1-4; photoreceptors R1-4. Bars = 20 μm (a, b, and c), 1 μm (**d** and **e**).

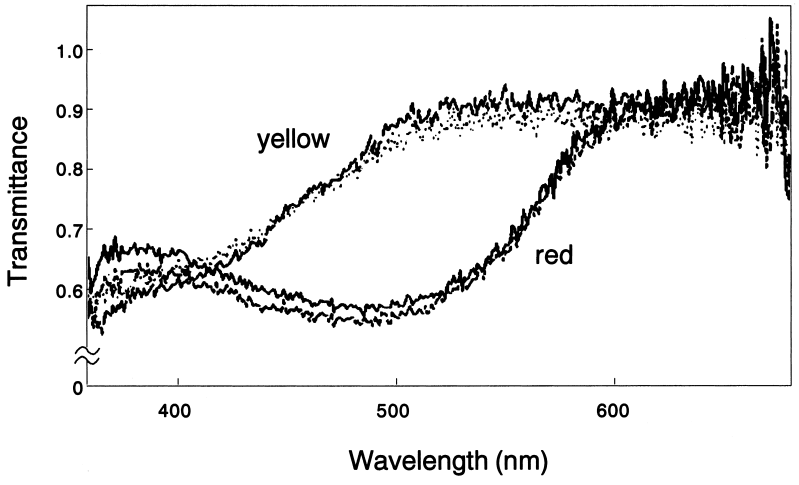

Fig. 4. Typical transmittance spectra of the colored screening pigments, measured from pigment clusters in frozen sections of unfixed material. Two spectra for each pigment are the measures from different pigment clusters in the same section. The transmittance spectra were obtained by dividing the measured transmission spectra of the pigment regions by the illumination spectrum measured at a clear region outside the section.

were used in the model calculations. Because the section thickness was 6 to 10 μm we calculate that $\kappa_s = 0.07 \pm 0.02\ \mu m^{-1}$. This value is probably slightly high, because the spectral measurements were performed on concentrated pigment clusters. This conjecture is underscored by the calculations. We obtained satisfactory fits of the calculated sensitivity spectra with the experimentally determined spectra with $\kappa_s = 0.05\ \mu m^{-1}$, which was the lower border value for κ_s.

The lateral filtering effect of the dark purple pigment in R1,2 photoreceptors was ignored in the present calculation, because the pigment stretches only over a very short distance along the rhabdom, at its distal tip, and therefore its spectral filtering effect is minor.

Spectral sensitivity of the photoreceptors in the R- and Y-ommatidia

We recorded the spectral sensitivity of 38 single, proximal (R5-8) photoreceptors; 25 cells appeared to be red receptors and 13 were green receptors (Fig. 5). The polarization sensitivity (PS) was also estimated. When the PS was maximal in a direction diagonally to the sagittal (dorso-ventral) plane, indicating that the receptor was a R5-8 cell, then lucifer yellow was injected subsequent to the spectral measurements. The pigmentation of the ommatidium of the recorded receptor could then be identified in plastic sections. Fig. 5 presents examples of lucifer yellow-stained red and green receptors with their spectral sensitivities (solid lines). All red receptors stained in the present study were in R-ommatidia, and all green receptors were in Y-ommatidia. The obtained sensitivity spectra demonstrate that the proximal photoreceptors (R5-8) in the R-ommatidia are red receptors peaking at about 600 nm. The sensitivity spectra of the red receptor is considerably narrower than the absorption spectrum expected for a 600 nm peaking visual pigment (Stavenga *et al.*, 1993). The R5-8 of the Y-ommatidia have a normal sensitivity profile that roughly fits to the predicted profile of a visual pigment peaking at 520 nm.

Modeling the spectral sensitivities

The narrow-band spectral sensitivity of the red receptors in the R-ommatidia can be well simulated with the model by assuming that the R5-8 possess a visual pigment with peak absorption at 575 nm (Fig. 6a). The red pigment around the rhabdom selectively absorbs in the wavelength region below 580 nm and thus causes the sensitivity peak to shift to 600 nm from the absorbance peak of the visual pigment at 575 nm. Furthermore, it causes the narrowing of the α-band. The accompanying drop in the absolute sensitivity of the cell at

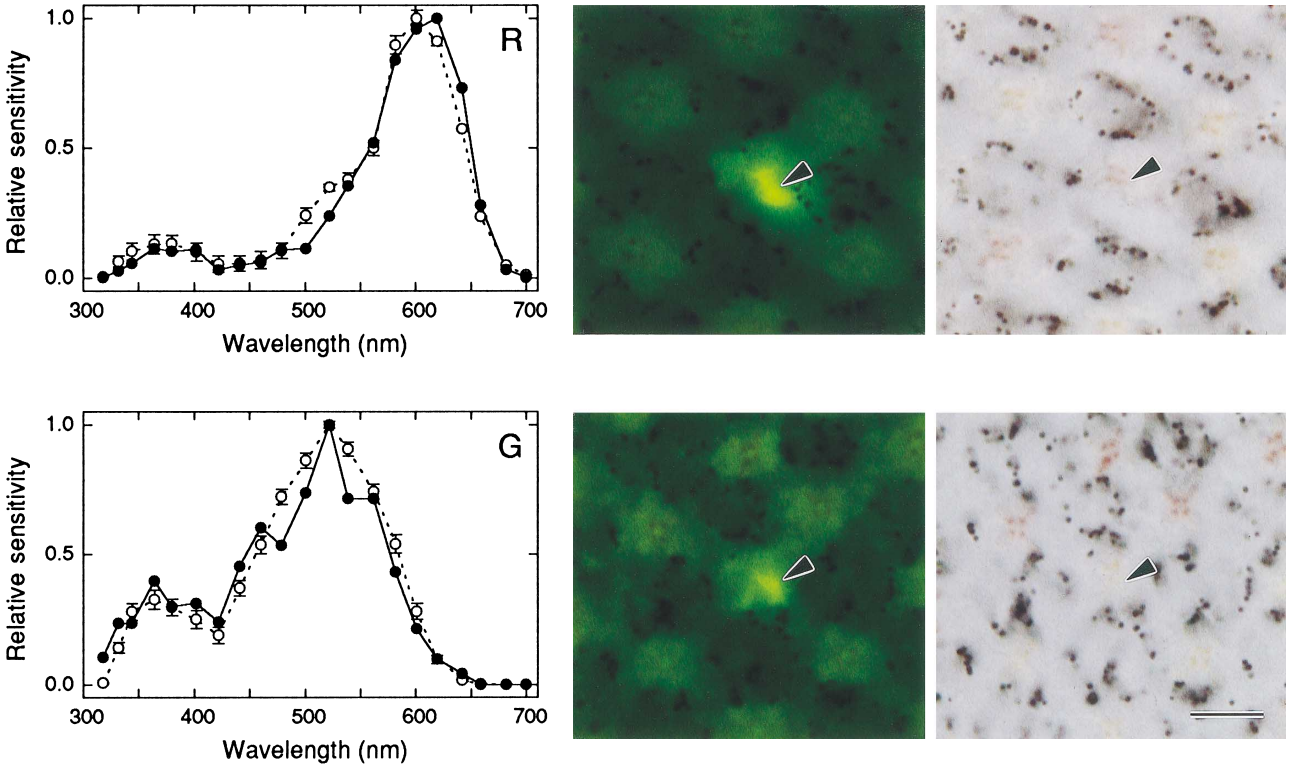

Fig. 5. Relation of spectral sensitivities of R5-8 and the pigmentation of ommatidia. The photoreceptors were stained with lucifer yellow after the spectral sensitivities were recorded, and then the eyes were further processed for light microscopy. The solid lines indicate the spectral sensitivity of the stained cells, whereas the dotted lines indicate the mean spectral sensitivity of each receptor type (after Bandai *et al.*, 1992). The stained photoreceptors were photographed in plastic sections under violet epi-illumination (middle). The same sections were also photographed under normal transmission (right). The histology shows that the red receptor (R) is a member of an R-ommatidium, and the green receptor (G) is in a Y-ommatidium (arrowheads). Bar = 10 μm.

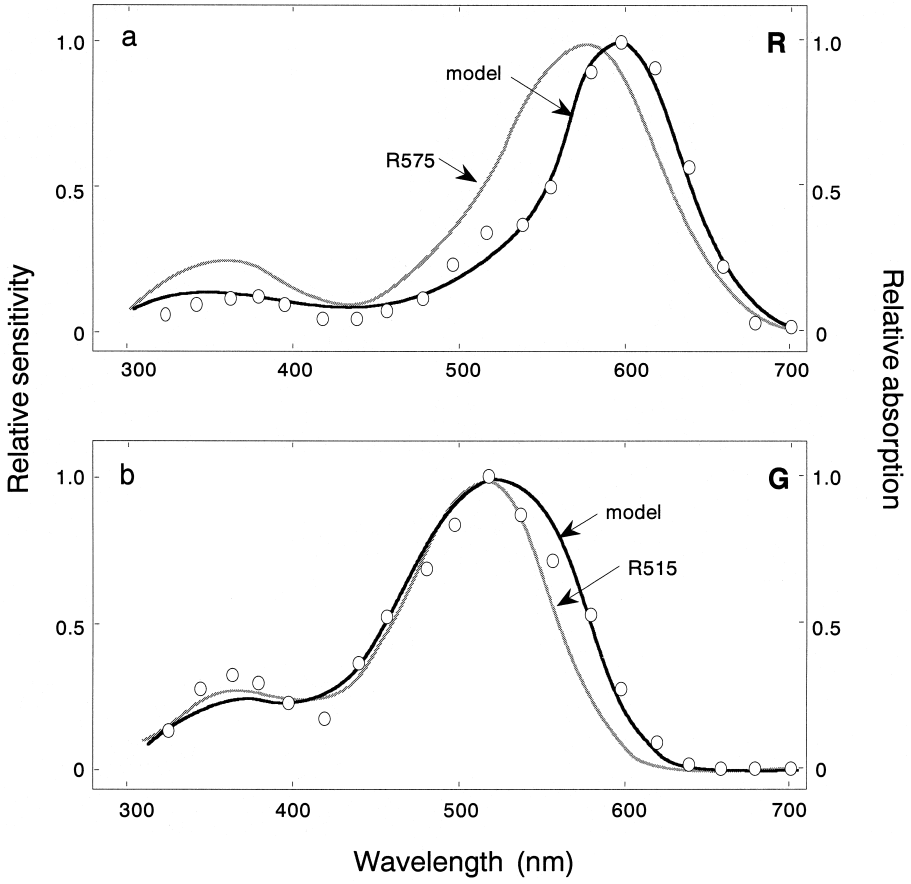

Fig. 6. Intracellularly determined spectral sensitivities fitted with an optical model for the *Papilio xuthus* ommatidium. Data points are those of Bandai *et al.* (1992) as in Fig. 5. **a** Red receptor. The red screening pigment causes a shift of the photoreceptor sensitivity spectrum with respect to the absorbance spectrum of the original visual pigment, peaking at 575 nm, resulting in a peak at 600 nm. **b** Green receptor. The yellow screening pigment causes a shift of the photoreceptor sensitivity spectrum with respect to the absorbance spectrum of the original visual pigment, peaking at 515 nm, resulting in a peak at 520 nm.

the peak wavelength was no more than 70 %.

The spectral sensitivity of the green receptors in the Y-ommatidia peaks at about 520 nm. It can be fitted by a visual pigment spectrum peaking at 520 nm. Yet, the yellow pigment will act as an optical filter, similar as the red pigment. When we assume that the red and yellow pigment have the same total optical density at short-wavelengths, then we estimate that the spectral sensitivities of the green receptors, peaking at 520 nm, result from a 515 nm visual pigment (Fig. 6b).

The results of the calculations were hardly affected by changing the visual pigment in the distal R1,2 photoreceptors, for example, from UV-absorbing to blue-absorbing type, or by adding/removing the UV-absorbing filter in the distal tier (Arikawa *et al.*, 1999).

DISCUSSION

Polymorphism of ommatidia

The purple pigmentation of the distal R1-2 cells resembles that of the Australian orchard butterfly *Papilio aegeus* (Horridge *et al.*, 1983). Also the pigmentation of the proximal photoreceptors of *Papilio xuthus* closely resembles the 'red pigmentation' observed in light-microscopical sections of the eye of *Papilio aegeus* (Ribi, 1987). Yet, at least in *Papilio xuthus* the proximal pigmentation appears to be heterogeneous, i.e. it is either red or yellow. The retina of *Papilio xuthus* is clearly polymorphic, in agreement with Bandai *et al.* (1992). Furthermore, polymorphism of ommatidia has been previously reported in other insects (digger wasp, *Sphex*: Ribi 1978b; fly, *Musca*: Franceschini *et al.*, 1981, Hardie *et al.*, 1981; moth, *Spodoptera*: Meinecke and Langer, 1984).

The polymorphic ommatidia are arranged in a random pattern. Randomness in the arrangement of different types of ommatidia is widely found among butterflies. By investigating unstained plastic sections of the retina, we discovered that differently colored ommatidia also occur in other papilionid species (*Parnassius glacialis*, *Papilio machaon*, *Papilio protenor*, *Papilio bianor*, *Papilio glaucus*, *Graphium sarpedon*), and furthermore in pierids (*Pieris rapae*, *Colias erate*), satyrids (*Neope goschkevitschii*, *Ypthima argus*), and lycaenids (*Zizeeria maha*, *Cretis acuta*, *Lycaena phlaeas*). In all cases, the distribution of the different types of ommatidia is random (see also Arikawa and Stavenga, 1997; Bernard and Miller,

1970). The anatomical studies clearly indicate that the different types of ommatidia will contain different types of spectral receptors.

In the distal tier, the R3 and R4 in all ommatidia are green receptors, regardless of the coloration (Bandai *et al*., 1992). What could be the functional reason? A clue can be found in the exemplary duplex retina of the common houseflies and blowflies. It consists of a homogenous set of highly sensitive, broad-band photoreceptors, R1-6, in all ommatidia, together with a heterogeneous set of pairs of less light sensitive, narrow band receptors, R7,8, sensitive in the UV and blue, and in the UV and blue-green, respectively (Hardie 1986). Whereas the R1-6 receptors mainly function in spatial information processing, the R7,8 photoreceptor pairs rather function in color discrimination (Troje, 1993). Possibly, therefore, the R3,4 (green) system of *Papilio* is designed for spatial vision, whereas the proximal R5-8 (green/red) system, together with the distal R1,2 (UV-violet-blue) receptors, are mainly involved in color vision.

Function of red pigment

The spectral sensitivity of butterfly red receptors can be quantitatively understood by assuming that light absorption by the visual pigment is distinctly affected by the clusters of pigment granules near the rhabdom, thus absorbing short-wavelength light (e.g. fly, Hardie *et al*., 1979; cabbage butterfly, Steiner *et al*., 1987; rev. Stavenga, 1989). The red pigment adjacent to the rhabdom distinctly reduces the sensitivity in the shorter wavelength range, so that the spectral band is narrowed and the peak is shifted to 600 nm. The same explanation was made before for the red receptor of the cabbage butterfly, *Pieris rapae* (Ribi, 1979). Similar lateral filters are found in the eye of stomatopod crustaceans, which bears intrarhabdomeral filters as well (Marshall *et al*., 1991). Both filtering systems effectively narrow the spectral sensitivities. Obviously, having red receptors with a narrow-band spectral sensitivity is both advantageous for expanding a butterfly's visual range and for improving wavelength discrimination.

A shift in spectral sensitivity due to the action of a pupillary pigment has been reported in the fly *Musca* (Hardie, 1979), i.e., the spectral sensitivity profile changes with the state of adaptation. When the fly photoreceptors R1-6 are light adapted, the sensitivity in the green region is selectively reduced, which is attributed to the light-dependent migration of pigment that only absorbs the boundary wave in the light adapted state. The essential function of the migratory pigment of fly photoreceptors is to control the light flux in the photoreceptors, i.e. to act as a so-called pupil. In *Papilio*, however, the spectral sensitivities of the proximal receptors in the dark- and light-adapted state were essentially identical (unpublished results). This suggests that the red and yellow pigment granules in *Papilio* do not move substantially, and that they remain near the rhabdom, even in darkness. This means that the red and yellow pigment systems in *Papilio* function as a stable color filter, rather than as a pupil.

In the modeling we assumed that light is propagated only in the lowest order waveguide mode. Probably this assumption is an oversimplification. For an analysis which includes higher order modes we have to know the relative light power of the various modes, which is difficult to assess. Furthermore, the outcome will not essentially affect our conclusions that the screening pigments act as short-wavelength absorbing filters.

Due to the limits of electrophysiology, we were unable to identify the spectral type of more than one of the four proximal photoreceptors per ommatidium. In the simulations, we have assumed that all proximal photoreceptors were of the same class. To our reassurance, recent *in situ* hybridization experiments with mRNAs encoding visual pigment opsins of *Papilio* underscore this hypothesis. It appeared that opsin mRNA probes either detected all four proximal R5-8 photoreceptors in an ommatidium, or none of them (Kitamoto *et al*., 1998). Of course, it will be of great interest to express the visual pigment clones and measure the absorbance spectra of the visual pigments *in vitro*, so to obtain an independent estimate of the visual pigment absorbance maxima.

By histological, electrophysiological, and optical experiments, we have so far identified three different types of ommatidia, randomly distributed over the retina (Arikawa and Stavenga, 1997). However, many uncertainties still remain: there may be more types of ommatidia. For instance, the visual pigments expressed in R1, 2 have not yet been cloned. A further unknown is created by the surprising finding that most likely in some photoreceptor cell types more than one visual pigment is expressed (Kitamoto *et al*., 1998). Possible consequences of the double expression for the spectral sensitivities have yet to be investigated. Nevertheless, the rich repertoire of photoreceptor types, as well as the sophisticated optics applied to realize the different cell types, suggest that spectral analysis is at a premium for *Papilio* butterflies. Indeed, recent behavioral studies on rapid color discrimination learning (Kinoshita *et al*., 1999) indicate that color vision is highly prized in *Papilio*.

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