J Comp Physiol A (2003) 189: 791–800
DOI 10.1007/s00359-003-0454-7

REVIEW

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Spectral organization of the eye of a butterfly, *Papilio*

Received: 2 June 2003 / Revised: 22 August 2003 / Accepted: 22 August 2003 / Published online: 30 September 2003

Abstract This review outlines our recent studies on the spectral organization of butterfly compound eyes, with emphasis on the Japanese yellow swallowtail butterfly, *Papilio xuthus*, which is the most extensively studied species. *Papilio* has color vision when searching for nectar among flowers, and their compound eyes are furnished with six distinct classes of spectral receptors (UV, violet, blue, green, red, broadband). The compound eyes consist of many ommatidia, each containing nine photoreceptor cells. How are the six classes of spectral receptors arranged in the ommatidia? By studying their electrophysiology, histology, and molecular biology, it was found that the *Papilio* ommatidia can be divided into three types according to the combination of spectral receptors they contain. Different types of ommatidia are distributed randomly over the retina. Histologically, the heterogeneity appeared to be related to red or yellow pigmentation around the rhabdom. A subset of red-pigmented ommatidia contains 3-hydroxyretinol in the distal portion, fluorescing under UV epi-illumination. The red, yellow and fluorescing pigments all play crucial roles in determining the spectral sensitivities of receptors. Spectral heterogeneity and random array of ommatidia have also been found in other lepidopteran species. Similarities and differences between species are also discussed.

Keywords Color vision · Compound eye
Photoreceptor · Retina · Rhabdom

Spectral receptors in butterfly eyes

When searching for nectar, butterflies find their sources, the flowers, by using their capacity for color vision (Kelber and Pfaff 1999; Kinoshita and Arikawa 2000; Kinoshita et al. 1999) in a way similar to that of honeybees (von Frisch 1914). The color information is, of course, detected by the compound eyes. The human color vision system is trichromatic, and is based on the short-, middle- and long-wavelength cone photoreceptor cells in the retina. Honeybees also have a trichromatic system with three types of spectral receptors in the eye, each peaking in the ultraviolet (UV), blue and green wavelength regions (Menzel and Backhaus 1989). As predicted from the spectral receptor types, honeybees are highly sensitive in the UV, but their sensitivity to red light is weak (von Frisch 1914); in fact, red flowers are not particularly attractive to honeybees. However, a number of butterfly species frequently visit red flowers. This observation raises several questions: (1) do butterflies recognize red?; (2)what type of spectral receptors do they possess?; and (3) how many types are there?

The compound eye consists of several thousands of units—the ommatidia. Figure 1 is a diagram of an ommatidium of the Japanese yellow swallowtail butterfly, *Papilio xuthus*, which is the species I will treat here as the model case. A single ommatidium of *Papilio*, like that of many other butterfly species, contains nine photoreceptor cells, R1–9. The photoreceptors bear straight and parallel microvilli towards the center of the ommatidium. The closely packed microvilli together construct a phototransductive rhabdom whose diameter is around 2.5 μm. Four cells, R1–4, are called distal photoreceptors, because they contribute their microvilli in the distal two-thirds of the rhabdom. The proximal one-third is made up with four proximal photoreceptors, R5–8. R9, the basal photoreceptor, adds some microvilli at the bottom of the ommatidium, immediately distal to the basement membrane.

To unravel the color vision system of butterflies, we first studied the spectral sensitivities of the photoreceptors by intracellular recording. After impaling a single photoreceptor with a glass microelectrode, we stimulated it with a series of monochromatic lights and recorded the evoked receptor potentials. We thus identified six types

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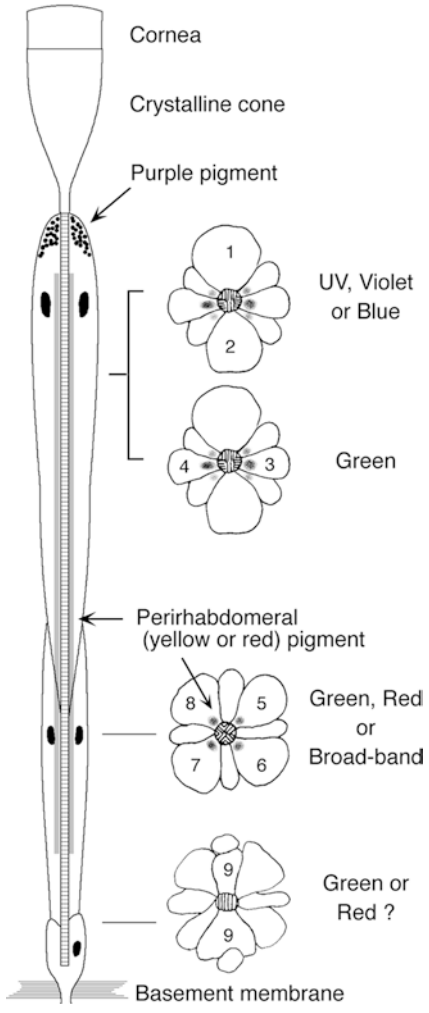

Fig. 1 Structure of the *Papilio* ommatidium: longitudinal (*left*) and transverse views of three different levels (*right*). The distal two-thirds of the rhabdom is made up with four distal photoreceptors (1–4), whereas four proximal photoreceptors (5–8) together construct the proximal one-third of the rhabdom. A basal photoreceptor (9) contributes to the rhabdom at the base of the ommatidium. Terms of colors on the right-hand side indicate the spectral type of the numbered photoreceptors

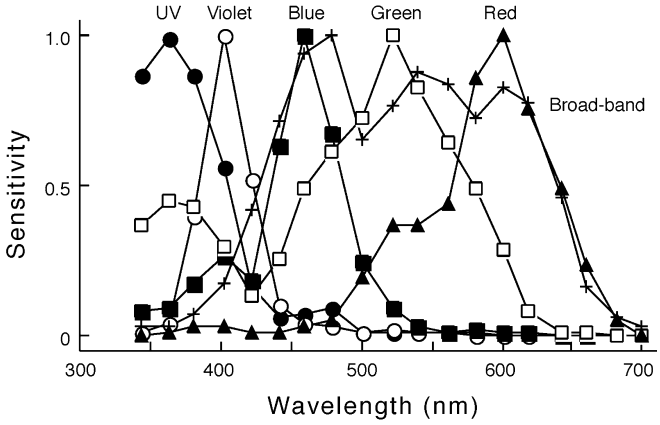

Fig. 2 Normalized spectral sensitivities identified in the *Papilio* retina. Six different types of spectral receptors were identified electrophysiologically. Five of them peak in the UV (*filled circle*), violet (*open circle*), blue (*filled square*), green (*open square*) and red (*filled triangle*) wavelength regions, respectively. The broadband type (*cross*) has no obvious peak

of spectral receptors in the *Papilio* retina (Fig. 2). Five of them express distinct peak sensitivities in the UV ($\lambda_{max} = 360$ nm), violet (400 nm), blue (460 nm), green (520 nm), and red (600 nm) wavelength regions, respectively (Arikawa et al. 1987). The sixth, anomalous type is a receptor with a very broadband spectral sensitivity, covering almost the whole (human) visible wavelength range (Arikawa et al. 2003).

We determined the anatomical identity of the spectral receptors by a conventional method of dye injection: we injected lucifer yellow into a penetrated photoreceptor after recording its spectral sensitivity, and then identified the location of the marked cell by histology. We also used a method we call the polarization method. Photoreceptors in the *Papilio* ommatidia bear parallel microvilli, making the photoreceptors polarization sensitive (Moody and Parriss 1961). For example, the microvilli of the *Papilio* R1 and R2 are oriented parallel to the dorso-ventral axis of the eye (see Fig. 1), and therefore these photoreceptors are maximally sensitive to polarized light with e-vector angle parallel to the dorso-ventral axis. By knowing the anatomical organization of the microvilli of the different photoreceptors we thus can determine the identity of a photoreceptor from its polarization sensitivity. After recording the spectral sensitivity, we subsequently recorded the polarization sensitivity to localize the penetrated photoreceptor in the ommatidium. Suppose that the polarization sensitivity of a UV receptor peaks at the angle parallel to the dorso-ventral axis: it means that the UV receptor is either an R1 or an R2. Of course, the polarization method is not applicable to eyes whose rhabdoms are twisted, as in bees and ants (Menzel and Blakers 1975).

The electrophysiological results revealed that the anatomical receptor types do not always have unique spectral properties (summarized in Fig. 1). Note for instance that R1 and R2 are either UV, violet, or blue receptors. Assigning three spectral receptors to only two anatomically distinct photoreceptors means that all ommatidia cannot be identical to each other in terms of the combination of the spectral receptors contained. In other words, the ommatidia must be spectrally heterogeneous.

Colors of ommatidia

The ommatidial heterogeneity can be directly observed by light microscopy. Figure 3a shows a transverse section through the proximal tier stained with the dye azur II: all the ommatidia look similar. However, a striking difference between ommatidia emerged when we observed the sections before staining (Fig. 3b): each ommatidium has four spots around the rhabdom. The

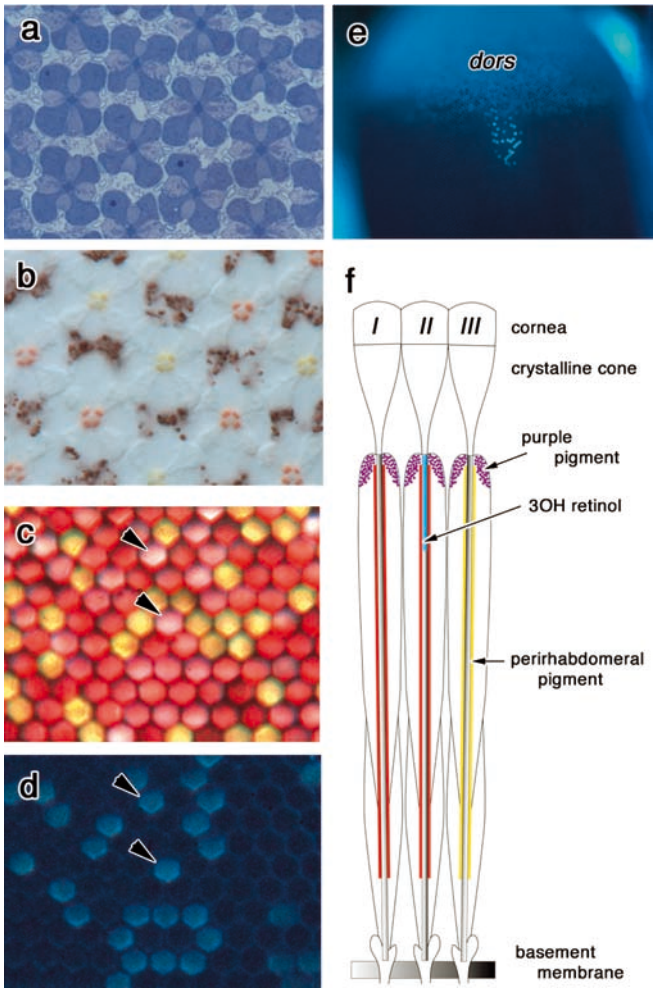

Fig. 3a–f Histological evidence for ommatidial heterogeneity in *Papilio*. **a** Transverse section of the *Papilio* retina through the proximal tier, stained with azur II. All ommatidia appear basically the same. **b** Unstained section of plastic-embedded specimen. Each ommatidium bears four spots of yellow or red pigment around the rhabdom. **c** Isolated fresh section of a compound eye illuminated from the proximal side, i.e., from the cut surface, and observed from the corneal side. Each ommatidium appears either yellow or (more or less saturated) red, corresponding to the colors of pigment lining the rhabdoms. **d** The same specimen as **c**, photographed using a fluorescence microscope with ultraviolet epi-illumination. Ommatidia of less saturated red emit strong fluorescence (*arrowheads* in **c** and **d**). **e** Low-magnification fluorescence picture of the *Papilio* eye, showing corneal fluorescence in the dorsal region (*dors*) and scattered ommatidial fluorescence in the ventral region. The limited area containing the fluorescing ommatidia is due to the small aperture of the objective lens. **f** Diagram of the three types of ommatidia: deep-red pigmented (*I*), red pigmented and fluorescing (*II*), and yellow pigmented (*III*). Type II has fluorescing 3-hydroxyretinol in the distal portion of the ommatidium

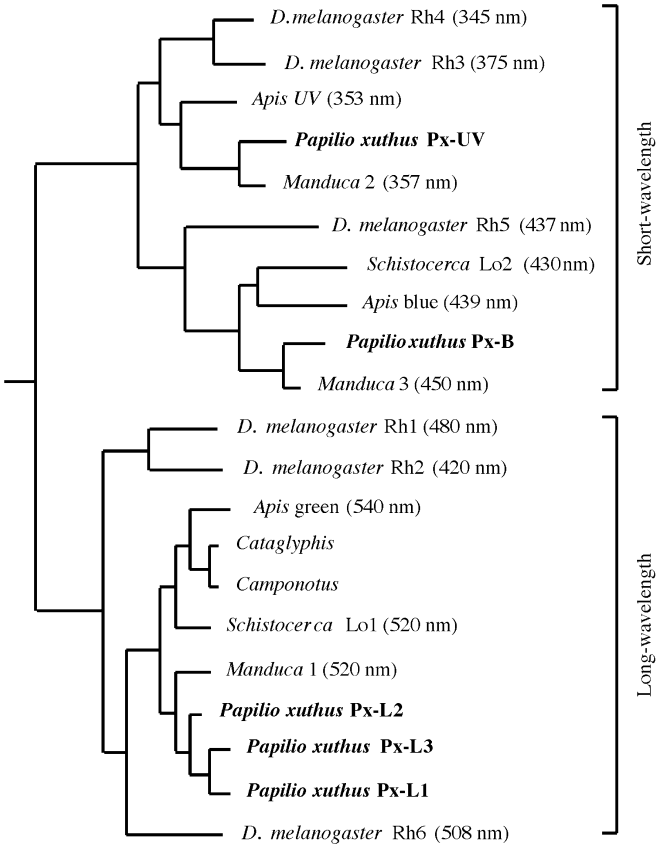

Fig. 4 Phylogeny of insect visual pigment opsins calculated using the neighbor-joining method with octopus opsin as the outgroup. Wavelength values indicate the peak wavelength of the visual pigment absorption spectra estimated by intracellular electrophysiology or by ERG measurement of transgenic *Drosophila* in which the corresponding opsins were expressed

spots, which are either yellow or red colored, are clusters of pigment granules located close to the rhabdom boundary in the cell body of the R5–8 photoreceptors. The ratio of the red-pigmented and the yellow-pigmented ommatidia is about 3:1. R3 and R4 also contain the red or yellow pigments, but R1 and R2 have purple pigment instead in the distal region in all ommatidia (see Figs. 1 and 3f).

What is the function of the pigments? The rhabdom, whose diameter is about 2.5 μm, acts as an optical waveguide because of its slenderness (Nilsson et al. 1988; van Hateren 1989). The red and yellow pigments, positioned within 1 μm of the rhabdom boundary, will absorb light propagated in the boundary wave, and thus function as spectral filters. The filtering effect can be directly observed in a simple experiment in which a fresh compound eye, sliced through the proximal tier, is illuminated from the cut surface and observed from the corneal side. The ommatidia then appear either yellow or (more or less saturated) red (Fig. 3c), corresponding to the color of the pigments surrounding the rhabdom. The color of the purple pigment of R1 and R2 is not observed this way, as it probably serves as a light-controlling, pupillary pigment (Stavenga 1979), although experiments to prove this have not been reported so far.

The less saturated red or whitish transmitting ommatidia (Fig. 3c) are a variant of the deeply red-pigmented ommatidia containing a low amount of red pigment. Most interestingly, these ommatidia emit a strong, whitish fluorescence under UV epi-illumination (Fig. 3d). The fluorescing material is 3-hydroxyretinol concentrated in the distal portion of the fluorescing ommatidia (Arikawa et al.

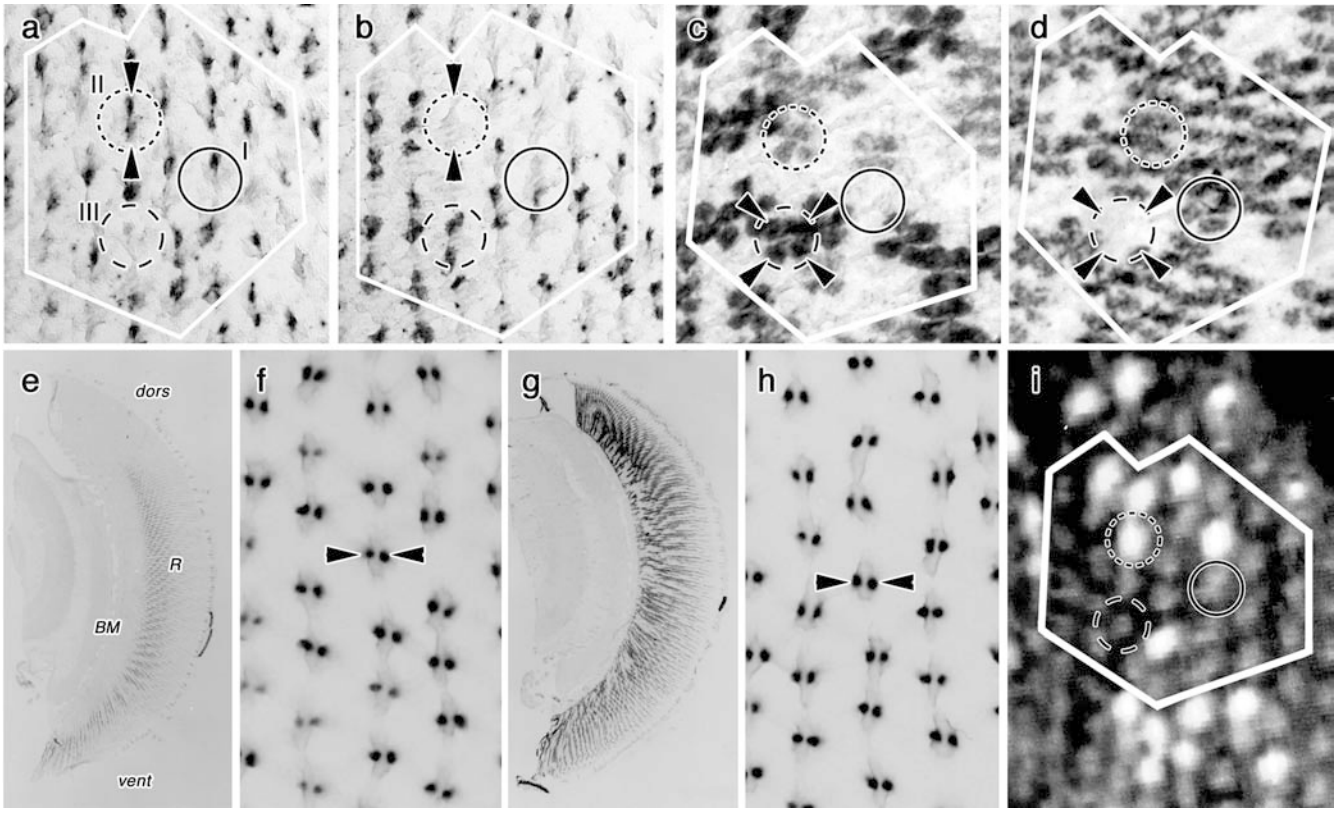

Fig. 5a–i Histological in situ hybridization. Sections in **a**, **b**, **c**, **d** and **i** were selected from a series of cryostat transverse sections from a single eye: *white polygons* indicate the same set of ommatidia in these sections. The set of ommatidia contains all three types of ommatidia (type I, *solid circle*; type II, *dotted circle*; type III, *dashed circle*). **a** In situ hybridization using the PxUV probe. A transverse section through the distal tier is shown. *Arrowheads* indicate R1 and R2. **b** Labeling with the PxB probe. Distal tier. **c** Labeling with the PxL2 probe. Proximal tier. *Arrowheads* indicate R5–8. **d** Labeling with the PxL3 probe. Proximal tier. **e** A longitudinal section labeled with the PxL1 probe. R, retina; BM, basement membrane; *dors* dorsal; *vent* ventral. **f** A transverse section through the distal tier labeled with the PxL1 probe. *Arrowheads* indicate R3 and R4. **g** Labeling with the PxL2 probe. Longitudinal section. **h** Labeling with PxL2 probe. Transverse section. **i** UV-induced fluorescence of a transverse section through the most distal part of the eye, taken before performing the in situ hybridization protocol. Fluorescing ommatidia correspond to type II ommatidia

1999a). About 25% of the ommatidia in the ventral half are of the fluorescing type. The ommatidial fluorescence is not observed in the dorsal half, because there the cornea itself fluoresces, meaning that the ommatidial fluorescence is either absent or obscured by the corneal fluorescence (Fig. 3e). (The border between the fluorescing and non-fluorescing cornea roughly corresponds to the equator of the visual field, which is why we refer to these regions as the dorsal 'half' and the ventral 'half'.)

Taken together, the *Papilio* compound eye contains at least three types of ommatidia (Fig. 3f), which are deep red-pigmented (type I), red-pigmented and fluorescing (type II), and yellow-pigmented (type III). The three types of ommatidia distribute rather randomly, at least locally in the ventral half of the eye.

Differential expression of visual pigment opsins among the ommatidial types

Visual pigment activates the phototransduction cascade upon absorption of a photon. A visual pigment molecule consists of a protein opsin with a vitamin A aldehyde attached to it as the chromophore. In the case of butterflies, the chromophore is 3-hydroxyretinal (Seki et al. 1987). The spectral sensitivity of a photoreceptor is primarily determined by the absorption spectrum of the visual pigment expressed in the photoreceptor, which depends on the interaction of the chromophore and the opsin part of the visual pigment molecule. Accumulated data of amino acid sequences of insect opsins have indicated that they can be categorized into two major groups based on the absorption spectra of the constituted visual pigment, i.e., a short- and long-wavelength absorbing group (Fig. 4).

Do the six types of spectral receptors in the *Papilio* retina express different visual pigment opsins? In order to answer this particular question, we carried out a molecular biological analysis. However, the result was not particularly straightforward: using histological in situ hybridization we identified only five opsin mRNAs instead of six, and, more interestingly, we found sets of photoreceptors that coexpress more than one opsin mRNA. A molecular phylogenetic analysis allowed the characterization of these five opsins as PxUV (*P. xuthus* UV-absorbing), PxB (blue-absorbing), and PxL1–3 (long-wavelength-absorbing 1–3), respectively (Fig. 4).

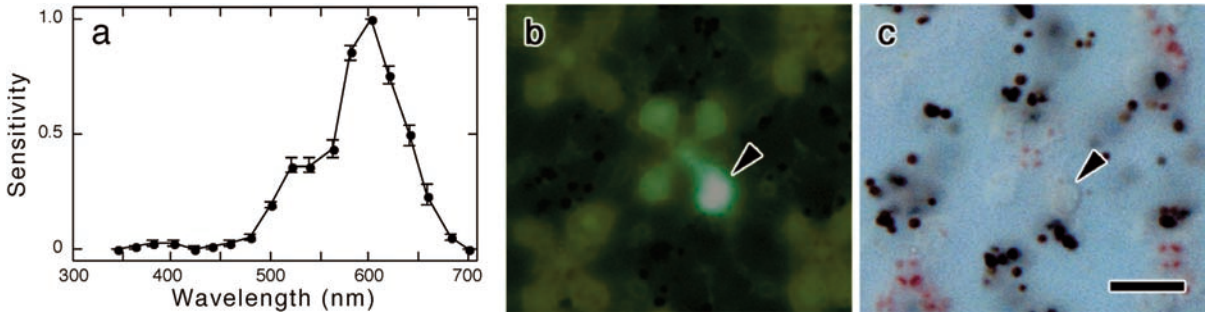

Fig. 6a–c Localization of a red receptor. **a** The spectral sensitivity of red receptors (bars = standard errors). **b** Blue-violet induced fluorescence of a section containing a lucifer yellow-injected red receptor. One of the proximal receptors (R6) was labeled (*arrowhead*). **c** The same section as **b**, observed in transmission with white light, indicating that the red-sensitive R6 is a member of a red-pigmented ommatidium. Scale: 10 μm

For histological in situ hybridization, we synthesized digoxigenin (DIG)-labeled cRNA probes for each opsin mRNA. We hybridized the probes to mRNAs on cryostat sections of the retina, and detected the hybridized probes by DIG immunohistochemistry (Kitamoto et al. 1998, 2000) (Fig. 5).

The probes for the mRNAs of PxUV and PxB exclusively labeled the R1 and R2, which are distal photoreceptors with vertically oriented microvilli (Fig. 5a, b). In type I ommatidia one of the R1 and R2 photoreceptors is labeled with the PxUV probe and the other with the PxB probe: when R1 is PxUV positive, R2 is PxB positive, and vice versa. R1 and R2 of type II are both labeled with the PxUV probe, and those of type III are labeled with the PxB probe. These results match the electrophysiological results: R1 and R2 are short wavelength receptors (Fig. 1).

The PxL1 probe labeled the horizontally oriented distal photoreceptors R3 and R4 only in the ventral half of the retina (Fig. 5e, f). The PxL1 is probably the opsin of a green-absorbing visual pigment, since these photoreceptors are green sensitive (Fig. 1).

The PxL2 probe labeled R3 and R4 in all ommatidia in both the dorsal and ventral halves (Fig. 5g, h). As R3 and R4 are green sensitive, PxL2 must also be an opsin of a green-absorbing visual pigment. The labeling of R3 and R4 in the ventral half by both PxL1 and PxL2 mRNAs suggest the hypothesis that these photoreceptors coexpress both PxL1 and PxL2 visual pigments (Fig. 5f, h).

The labeling pattern of PxL2 and PxL3 in the proximal R5–8 photoreceptors appears to be even more complicated. R5–8 in the type I ommatidia were labeled exclusively with the PxL3 probe (Fig. 5d), whereas those of type III were exclusively labeled with the PxL2 probe (Fig. 5c). Interestingly, R5–8 of type II ommatidia, which fluoresce under UV (Fig. 5i), were double labeled by both the PxL2 and PxL3 probes (Fig. 5c, d).

Spectral tuning and the ommatidial types

The spectral sensitivities of *Papilio* photoreceptors (Fig. 2) cannot be simply explained by the absorption spectra of visual pigments (Govardovskii et al. 2000). For example, the sensitivity of violet receptors, peaking at 400 nm, is much too narrow compared to the predicted spectrum of a visual pigment absorbing maximally at 400 nm (see Fig. 8). Furthermore, if the colocalized multiple visual pigment mRNAs express functional visual pigments, this should result in char-

Table 1 Summary of the characteristics of the three ommatidial types in *Papilio xuthus*

Type of ommatidia	Type I		Type II		Type III	
Ratio	50%		25%		25%	
Pigmentation	Red		Red		Yellow	
Fluorescence	No		Yes		No	
Photoreceptors	$S(\lambda)$	*Opsin*	$S(\lambda)$	*Opsin*	$S(\lambda)$	*Opsin*
R1	UV*	PxUV*	V	PxUV	B	PxB
R2	B*	PxB*	V	PxUV	B	PxB
R3–4	DG	PxL1 + L2	SG	PxL1 + L2	DG	PxL1 + L2
R5–8	R	PxL3	BB	PxL2 + L3	DG	PxL2
R9	?	PxL1 + L2	?	PxL1 + L2	?	PxL1 + L2

In addition to the perirhabdomeral pigmentation and the autofluorescence, the spectral sensitivity $S(\lambda)$ of the photoreceptors is given and the visual pigment opsins expressed in the ventral half of the eye

The sensitivity spectrum of R9 photoreceptors is an open question; it probably peaks in the orange wavelength range, but electrophysiological data are lacking so far. For the $S(\lambda)$ of DG and SG receptors see Arikawa et al (1999a) and for those of other receptors see Fig. 2. For type I, the spectral type of R1 and R2 may be interchanged (*asterisk*)

UV ultraviolet; *V* violet; *B* blue; *DG* double-peaked green; *SG* single-peaked green; *R* red; *BB* broad-band

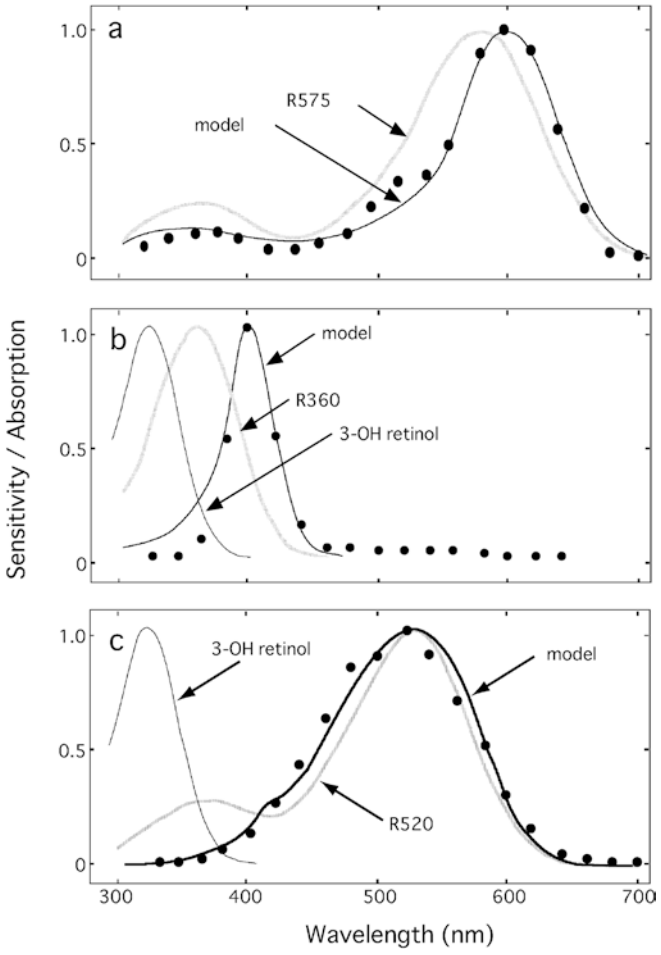

Fig. 7a–c Intracellularly determined spectral sensitivities (filled circles) fitted with an optical model for the *Papilio* ommatidium. **a** Red receptor. The red screening pigment causes a shift of the spectrum with respect to the absorbance spectrum of the original visual pigment, peaking at 575 nm, resulting in a peak at 600 nm. **b** Violet receptor. Combining the effect of the UV-absorbing pigment (the fluorescing 3-hydroxyretinol) and the red screening pigment on the UV-absorbing visual pigment peaking at 360 nm causes the narrow spectral sensitivity of violet receptors. **c** Single-peaked green receptor. The UV-absorbing pigment in type II ommatidia significantly reduces UV sensitivity of green receptors, which are usually double peaked in the green- and UV-wavelength regions with a visual pigment peaking at 520 nm

acteristically modified spectral sensitivities. We studied the spectral tuning of photoreceptors in detail by postulating the optical interaction of the visual pigments with the red, yellow, and fluorescing pigments. The present understanding of the photoreceptor spectral tuning is reviewed below, with the focus on the red, violet, single-peaked green, and broadband receptors of *Papilio*.

Red receptors

By combining electrophysiology and histology, we first demonstrated that the red receptors are R5–8 photoreceptors located in red-pigmented, non-fluorescing (type I) ommatidia (Fig. 6). These photoreceptors express PxL3 mRNA, indicating that PxL3 constructs the opsin of a red-absorbing visual pigment (Table 1). The spectral sensitivity of the red receptors is rather narrow compared to the predicted absorption spectrum of a 600-nm peaking visual pigment (Stavenga et al. 1993). The difference can be explained by the filtering effect of the red pigment on the boundary wave. To provide a quantitative description of the spectral sensitivity, we constructed a computer simulation model encompassing all the known anatomical details of the ommatidial structure. Then we predicted the spectral sensitivity of R5–8 by varying the peak absorption wavelength of the visual pigments in each of the photoreceptors of type I ommatidia as well as the optical densities of the visual pigments and the red screening pigment. The spectral sensitivity of the red receptors can be reproduced when we incorporate a 575-nm peaking visual pigment into R5–8 photoreceptors with a suitable density (Fig. 7a). Similarly, we found that the green sensitive R5–8 photoreceptors are always located in type III ommatidia. The model prediction fitted reasonably to the recorded sensitivity when we incorporated a 515-nm visual pigment in these cells (see Arikawa et al. 1999b).

Violet receptors

Violet receptors also have an aberrantly narrow sensitivity spectrum (Fig. 2). Here, the fluorescing pigment, 3-hydroxyretinol, plays a crucial role. We extended the above simulation model for the type II ommatidia with the UV absorbing 3-hydroxyretinol incorporated in the distal tier (Arikawa et al. 1999a). Assuming that the R1 and R2 distal photoreceptors contain UV-absorbing visual pigment, and that the distally concentrated 3-hydroxyretinol acts as a UV-absorbing spectral filter for the photoreceptors, the model could predict a spectral sensitivity of R1 and R2 closely resembling the recorded spectrum of the violet receptors (Fig. 7b). The model predicted also that the violet receptors must exist in the fluorescing type II ommatidia and that the R1 and R2 of type II ommatidia must contain a UV-absorbing visual pigment. The second point has already been confirmed by in situ hybridization: R1 and R2 in type II ommatidia share with R1 or R2 in type I ommatidia an mRNA that encodes a UV visual pigment (Fig. 5a). To test the first point, we carried out an electrophysiological experiment where we injected lucifer yellow into a violet receptor, and then localized the ommatidium containing the marked violet receptor by fluorescence microscopy. After photographing the lucifer yellow fluorescence under violet excitation, we switched the excitation light to UV to visualize the fluorescence of 3-hydroxyretinol. We thus found without exception that the violet receptor-containing ommatidia are the fluorescing type II ommatidia (Fig. 8). These type II ommatidia must, of course, bear red pigment around the rhabdom (Fig. 3d), which was confirmed by subsequent histology. We then

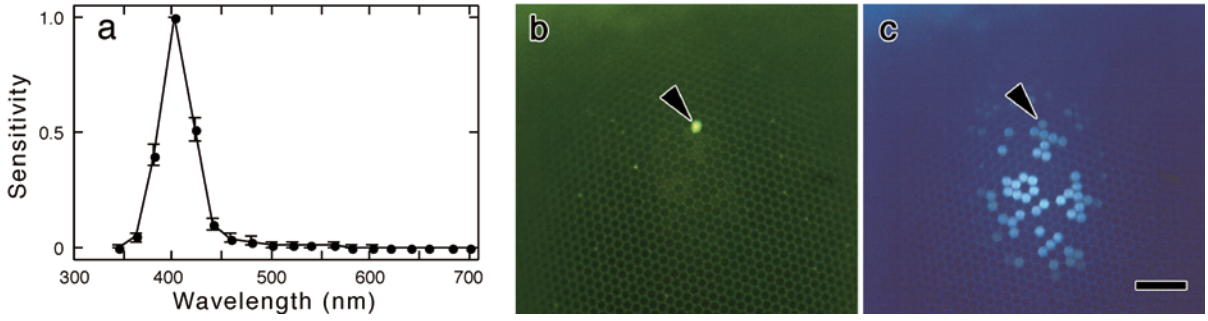

Fig. 8a–c Localization of a violet receptor. **a** The spectral sensitivity of violet receptors (bars = standard errors). **b** Blue-violet induced fluorescence of the whole compound eye containing a lucifer yellow-injected violet receptor. The ommatidium containing the violet receptor fluoresces (*arrowhead*). **c** The same eye as **b**, taken under UV epi-illumination, demonstrating that the ommatidium is a fluorescing type II ommatidium. Scale: 100 μm

localized UV receptors with the same method: UV receptors exist exclusively in non-fluorescing type I ommatidia (data not shown).

Single-peaked green receptors

The UV absorbing function of 3-hydroxyretinol of course affects the spectral sensitivities of R3–8 photoreceptors in type II ommatidia. R3 and R4 are green sensitive in all ommatidia, which is confirmed by both electrophysiology (Fig. 1) and in situ hybridization (Figs. 5f, h). Although the majority of green receptors are double-peaked with a secondary peak in the UV (Fig. 2), we sometimes encountered single-peaked green receptors with very reduced UV sensitivity (Bandai et al. 1992). We later revealed that the single-peaked green receptors actually exist in the type II ommatidia (Arikawa et al. 1999a). The reduction of UV sensitivity can be explained by the pre-absorption of UV light by 3-hydroxyretinol at the distal portion of the type II ommatidia (Fig. 7c).

Broadband receptors

The broadband receptors are quite unique among animal photoreceptors. The broadband spectral sensitivity has a half-bandwidth of about 210 nm with no obvious peak (Fig. 1). We localized the broadband receptors as R5–8 of type II ommatidia by intracellular penetration and dye injection (Fig. 9): these photoreceptors coexpress PxL2 and PxL3 mRNAs (Fig. 5c, d). We were able to describe the spectral sensitivity with the model for the red screening pigment containing type II ommatidia by incorporating the 515-nm visual pigment (PxL2) as well as the 575-nm visual pigment (PxL3) in the R5–8 photoreceptors (Fig. 9a).

The combination of the various experimental approaches, namely anatomy, electrophysiology and molecular biology, combined with the modeling, has

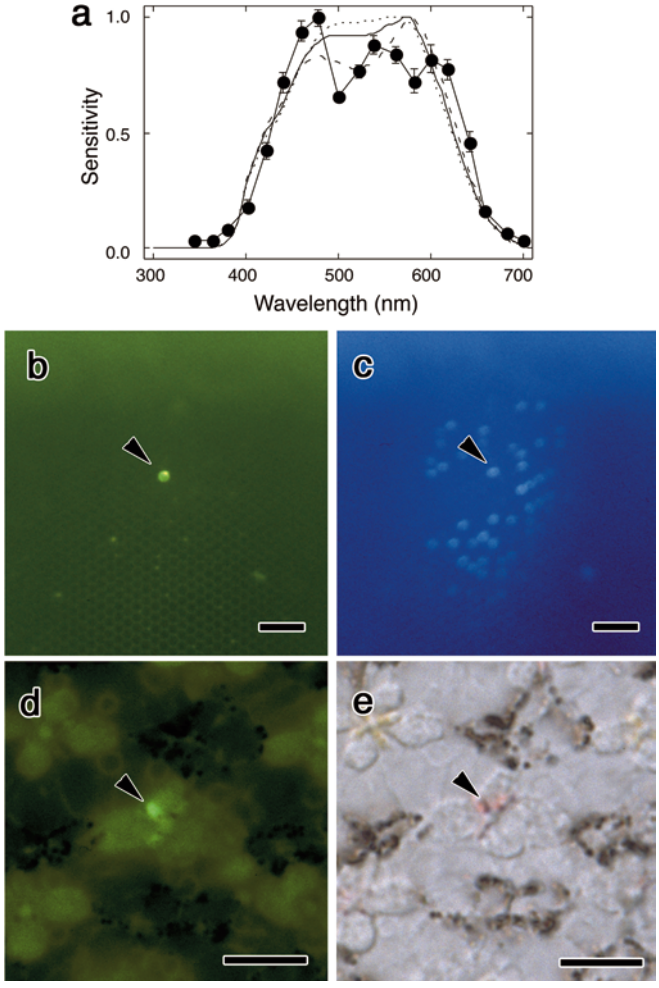

Fig. 9a–e Localization of a broadband receptor. **a** The spectral sensitivity of broadband receptors (*filled circle with standard error bars*), with the model-calculated spectra assuming that the incident light is polarized, with the e-vector angle of 0° (*dotted curve*), 90° (*dashed curve*), or 35°/125° (*continuous curve*). **b** The ommatidium containing the lucifer-injected unit (*arrowhead*). **c** UV excitation showing that the ommatidium was of type II. **d** Section of the eye observed under violet excitation. The unit was a proximal photoreceptor, R8. **e** Regular transmission microscopy revealed that the ommatidium of the labeled photoreceptor contained red pigment. Scales: 100 μm in **b** and **c**, and 10 μm in **d** and **e**

yielded the insight that the *Papilio* retina is composed of three different types of ommatidia, each containing a unique set of spectral receptors (Table 1). The basis of the spectral sensitivities of the photoreceptors is some-

times quite complicated, and often due to the combined effect of more than one visual pigment and the filtering by photostable pigments. The finally resulting spectral sensitivities have presumably been realized to optimize color vision. Discussions about the evolution of color vision, usually focus exclusively on the spectral tuning of visual pigment molecules based on their amino acid sequences (Chittka and Briscoe 2001; Gaertner 2000; Pichaud et al. 1999; Yokoyama and Yokoyama 2000). However, several other factors may play a role, as is demonstrated by the photoreceptors of *Papilio*. A similar organization as in *Papilio*, where multiple visual pigments are expressed in single cells or where spectral filtering sharpens the spectral sensitivities, may be encountered in many other animals, both in vertebrates (Lyubarsky et al. 1999; Makino and Dodd 1996) and in invertebrates (Marshall et al. 1991; Sakamoto et al. 1996). Flies utilize a different mechanism to broaden the spectral sensitivity of the peripheral (R1–6) photoreceptors. The photoreceptors contain a blue sensitive visual pigment (Rh1) with one molecule of 3-hydroxyretinol attached as a photostable sensitizing pigment. The sensitizing pigment absorbs UV light and transfers the energy to Rh1, resulting in another high sensitivity in the UV wavelength region in addition to the main sensitivity band peaking at around 480 nm (Stavenga et al. 2000).

Comparative view

The *Papilio* retina is a random mesh of three types of ommatidia, at least in the ventral half of the compound eye. Is this specific to *Papilio*, or is this arrangement shared by other insects? The phenomenon of ommatidial heterogeneity itself was repeatedly reported in flies (Franceschini et al. 1981; Hardie et al. 1981; Chou et al. 1996), a digger wasp (Ribi 1978), and in a moth (Meinecke and Langer 1984). Ommatidial heterogeneity was demonstrated in many butterfly species by their characteristic tapetal reflection (Bernard and Miller 1970; Stavenga et al. 2001). Stavenga (2002a) developed wide-angle telescope optics to study the ommatidial heterogeneity of butterflies into great detail, and thus clearly demonstrated the dorso-ventral specialization and species diversity of reflection colors (Stavenga 2002b). However, the eyes of papilionid butterflies form an exception by lacking the tapetum, and thus they do not exhibit the phenomenon of tapetal reflection.

The study of lepidopteran eyes has recently seen considerable progress. The eyes of the small white *Pieris rapae* (Pieridae) (Qiu et al. 2002; Qiu and Arikawa 2003a), the painted lady *Vanessa cardui* (Nymphalidae) (Briscoe et al. 2003), and the nocturnal tobacco hawkmoth *Manduca sexta* (Sphingidae) (White et al. 2003) all appear to contain at least three types of ommatidia in the ventral half. The striking similarity among these species is found in the combination of short-wavelength receptors in one of the three types of ommatidia. Typically, one type has two UV receptors, one has two blue receptors, and one has both UV and blue receptors (see Table 1 for the case of *Papilio*). Colocalization of UV and blue receptors in a single ommatidium suggests that these animals are able, in principle, to discriminate slight wavelength differences of a very small target in the short wavelength region of the spectrum. Actually, a day-active sphingid *Macroglossum stellatrum* can discriminate two similar spectral lights in the UV region (e.g., 365 nm and 380 nm) upon feeding (Kelber and Henique 1999). This property is probably important for nectar-feeding insects. In fact, our preliminary data have shown that the same photoreceptor organization occurs in the honeybee *Apis mellifera* (K. Arikawa, unpublished observations).

Of course, species differences certainly exist. If we define red receptors as the receptors with a rather sharp sensitivity profile peaking in the wavelength region longer than ca. 600 nm, the R5–8 of type I ommatidia of *Papilio* qualify as a type of red receptor ($\lambda_{max} = 600$ nm, Fig. 2), but bees do not possess such a type of receptor (Menzel and Backhaus 1989). Do all butterflies have some type of red receptor? The answer must be no, for at least two reasons. Firstly, we have shown by electrophysiology that two species of nymphalid butterflies, *Polygonia c-aureum* and *Sasakia charonda*, do not have photoreceptors with spectral sensitivities peaking in the red; their L-receptors peak in the green to orange wavelength regions (Kinoshita et al. 1997). Recent microspectroscopical and molecular biological investigations on *Vanessa* revealed that their eyes express only three types of visual pigments, with probable absorption peaks at 360, 470, and 530 nm (Briscoe et al. 2003). The situation in the diurnal *Vanessa* is quite similar to that of the nocturnal *Manduca* (White et al. 2003). Secondly, there are species that have more than one type of red receptor: we have identified two distinct long-wavelength (red) receptors, LW620 and LW640, peaking at 620 nm and 640 nm, respectively, in the eye of the male small white, *Pieris rapae*, by intracellular electrophysiology and subsequent histology (Qiu and Arikawa 2003b). The *Pieris* eye consists of three types of ommatidia that are easily distinguished by their perirhabdomeral pigmentation (Qiu et al. 2002). The LW620 receptors exist in the proximal tier of type I and III ommatidia, whereas the LW640 receptors were found exclusively in the proximal tier of type II ommatidia. Type II ommatidia express a denser red pigmentation compared to that of type I and III ommatidia, which is a possible cause of the difference in the spectral sensitivities between the two types of red receptors. Whether the opsins expressed in these receptors are identical or not is still an open question.

Another notable difference, at least at the present stage, is that coexpression of multiple visual pigments in single photoreceptors is exclusively found in the *Papilio* eye. We clearly demonstrated in *Papilio* that the R5–8 in type II ommatidia contain two functional visual pigments with different absorption spectra, resulting in a very wide spectral sensitivity (Fig. 9) (Arikawa et al.

2003). Is this specific to *Papilio*, or is it common in other animals? This question must await further molecular biological analyses.

The biological significance of the ommatidial heterogeneity and the randomness is still enigmatic. Accumulated data have indicated that green receptors are present in all ommatidia forming a complete hexagonal lattice (e.g., R3 and R4 of *Papilio*, Table 1), whereas other receptors distribute rather randomly. The regular hexagonal array of green receptors of course favors good spatial resolution. The random system of several spectral receptor types (including green receptors of R5–8, see Table 1) may be related to color vision, perhaps being required to avoid chromatic aliasing. The retinal randomness is also shared by primates, which have sophisticated color vision systems: a random distribution of M- and L-cones has been demonstrated in talapoin monkeys (Mollon and Bowmaker 1992) and also in living humans (Roorda and Williams 1999). Is the randomness essential for recognizing color? Probably not, because some fish and mantis shrimp, which have very regular retinae, have true color vision (Marshall et al. 1996; Neumeyer 1992), although fundamental differences may exist between the color vision systems of aquatic and terrestrial dwellers. At any rate, the retina of butterflies provides an excellent subject for studying the functional significance of the receptor mosaic for color vision and for visual acuity in general.

Acknowledgements I first want to express my thanks to the editor of this journal for encouraging me to write this review. Drs D.G. Stavenga and M. Kinoshita, and two anonymous referees provided critical comments on the manuscript. The work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT) and by the PRESTO program of the Japan Science Technology Corporation (JST).

References

Arikawa K, Inokuma K, Eguchi E (1987) Pentachromatic visual system in a butterfly. Naturwissenschaften 74:297–298

Arikawa K, Mizuno S, Scholten DGW, Kinoshita M, Seki T, Kitamoto J, Stavenga DG (1999a) An ultraviolet absorbing pigment causes a narrow-band violet receptor and a single-peaked green receptor in the eye of the butterfly *Papilio*. Vision Res 39:1–8

Arikawa K, Scholten DGW, Kinoshita M, Stavenga DG (1999b) Tuning of photoreceptor spectral sensitivities by red and yellow pigments in the butterfly *Papilio xuthus*. Zool Sci 16:17–24

Arikawa K, Mizuno S, Kinoshita M, Stavenga DG (2003) Coexpression of two visual pigments in a photoreceptor causes an abnormally broad spectral sensitivity in the eye of a butterfly, *Papilio xuthus*. J Neurosci 23:4527–4532

Bandai K, Arikawa K, Eguchi E (1992) Localization of spectral receptors in the ommatidium of butterfly compound eye determined by polarization sensitivity. J Comp Physiol A 171:289–297

Bernard GD, Miller WH (1970) What does antenna engineering have to do with insect eyes? IEEE Student J 8:2–8

Briscoe AD, Bernard GD, Szeto AS, Nagy LM, White RH (2003) Not all butterfly eyes are created equal: rhodopsin absorption spectra, molecular identification and localization of UV-, blue-, and green-sensitive rhodopsin encoding mRNA in the retina of *Vanessa cardui*. J Comp Neurol 458:334–349

Chittka L, Briscoe AD (2001) Why sensory ecology needs to become more evolutionary—insect color vision as a case in point. In: Barth FG, Schmid A (eds) Ecology of sensing. Springer, Berlin Heidelberg New York, pp 19–37

Chou WH, Hall KJ, Wilson DB, Wideman CL, Townson SM, Chadwell LV, Britt SG (1996) Identification of a novel *Drosophila* opsin reveals specific patterning of the R7 and R8 photoreceptor cells. Neuron 17:1101–1115

Franceschini N, Kirschfeld K, Minke B (1981) Fluorescence of photoreceptor cells observed in vivo. Science 213:1264–1267

Frisch K von (1914) Der Farbensinn und Formensinn der Biene. Zool J Physiol 37:1–238

Gaertner W (2000) Invertebrate visual pigments. In: Stavenga DG, DeGrip WJ, Pugh ENJ (eds) Molecular mechanisms in visual transduction. Elsevier, Amsterdam, pp 297–388

Govardovskii VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K (2000) In search of the visual pigment template. Vis Neurosci 17:509–528

Hardie RC, Franceschini N, Ribi W, Kirschfeld K (1981) Distribution and properties of sex-specific photoreceptors in the fly *Musca domestica*. J Comp Physiol A 145:139–152

Hateren JH van (1989) Photoreceptor optics, theory and practice. In: Stavenga DG, Hardie RC (eds) Facets of vision. Springer, Berlin Heidelberg New York, pp 74–89

Kelber A, Henique U (1999) Trichromatic colour vision in the humming bird hawkmoth, *Macroglossum stellatarum* L. J Comp Physiol A 184:535–541

Kelber A, Pfaff M (1999) True colour vision in the orchard butterfly, *Papilio aegeus*. Naturwissenschaften 86:221–224

Kinoshita M, Arikawa K (2000) Colour constancy of the swallowtail butterfly, *Papilio xuthus*. J Exp Biol 203:3521–3530

Kinoshita M, Sato M, Arikawa K (1997) Spectral receptors of nymphalid butterflies. Naturwissenschaften 84:199–201

Kinoshita M, Shimada N, Arikawa K (1999) Colour vision of the foraging swallowtail butterfly *Papilio xuthus*. J Exp Biol 202:95–102

Kitamoto J, Sakamoto K, Ozaki K, Mishina Y, Arikawa K (1998) Two visual pigments in a single photoreceptor cell: identification and histological localization of three mRNAs encoding visual pigment opsins in the retina of the butterfly *Papilio xuthus*. J Exp Biol 201:1255–1261

Kitamoto J, Ozaki K, Arikawa K (2000) Ultraviolet and violet receptors express identical mRNA encoding an ultraviolet-absorbing opsin: Identification and histological localization of two mRNAs encoding short-wavelength-absorbing opsins in the retina of the butterfly *Papilio xuthus*. J Exp Biol 203:2887–2894

Lyubarsky AL, Falsini B, Pennesi ME, Valentini P, Pugh ENJ (1999) UV- and midwave-sensitive cone-driven retinal responses of the mouse: a possible phenotype for coexpression of cone photopigments. J Neurosci 19:442–455

Makino CL, Dodd RL (1996) Multiple visual pigments in a photoreceptor of the salamander retina. J Gen Physiol 108:27–34

Marshall NJ, Land MF, King CA, Cronin TW (1991) The compound eyes of mantis shrimps (Crustacea, Hoplocarida, Stomatopoda). 2. Colour pigments in the eyes of stomatopod crustaceans: polychromatic vision by serial and lateral filtering. Philos Trans R Soc Lond B 334:57–84

Marshall NJ, Jones JP, Cronin TW (1996) Behavioural evidence for colour vision in stomatopod crustaceans. J Comp Physiol A 179:473–481

Meinecke CC, Langer H (1984) Localization of visual pigments within rhabdoms of the compound eye of *Spodoptera exempta* (Insecta, Noctuidae). Cell Tissue Res 238:359–368

Menzel R, Backhaus W (1989) Color vision in honey bees: phenomena and physiological mechanisms. In: Stavenga DG, Hardie RC (eds) Facets of vision. Springer, Berlin Heidelberg New York, pp 281–297

Menzel R, Blakers M (1975) Functional organisation of an insect ommatidium with fused rhabdom. Cytobiology 11:279–298

Mollon JD, Bowmaker JK (1992) The spatial arrangement of cones in the primate fovea. Nature 360:677–679

Moody MF, Parriss JR (1961) The discrimination of polarized light by *Octopus*: a behavioral and morphological study. Z Vergl Physiol 44:268–291

Neumeyer C (1992) Tetrachromatic color vision in goldfish—evidence from color mixture experiments. J Comp Physiol A 171:639–649

Nilsson D-E, Land MF, Howard J (1988) Optics of the butterfly eye. J Comp Physiol A 162:341–366

Pichaud F, Briscoe A, Desplan C (1999) Evolution of color vision. Curr Opin Neurobiol 9:622–627

Qiu X, Arikawa K (2003a) The photoreceptor localization confirms te spectral heterogeneity of ommatidia in the male small white butterfly, *Pieris rapae crucivora*. J Comp Physiol A 189:81–88

Qiu X, Arikawa K (2003b) Polymorphism of red receptors: Sensitivity spectra of proximal photoreceptors in the small white butterfly, *Pieris rapae crucivora*. J Exp Biol 206:2787–2793

Qiu X, Vanhoutte KAJ, Stavenga DG, Arikawa K (2002) Ommatidial heterogeneity in the compound eye of the male small white butterfly, *Pieris rapae crucivora*. Cell Tissue Res 307:371–379

Ribi WA (1978) A unique hymenopteran compound eye. The retina fine structure of the digger wasp *Sphex cognatus* Smith (Hymenoptera, Sphecidae). Zool Jahrb Abt Allg Zool Physiol Tiere 100:299–342

Roorda A, Williams DR (1999) The arrangement of the three cone classes in the living human eye. Nature 397:520–522

Sakamoto K, Hisatomi O, Tokunaga F, Eguchi E (1996) Two opsins from the compound eye of the crab *Hemigrapsus sanguineus*. J Exp Biol 199:441–450

Seki T, Fujishita S, Ito M, Matsuoka N, Tsukida K (1987) Retinoid composition in the compound eyes of insects. J Exp Biol 47:95–103

Stavenga DG (1979) Pseudopupils of compound eyes. In: Autrum H (eds) Handbook of sensory physiology. Springer, Berlin Heidelberg New York, pp 357–439

Stavenga DG (2002a) Colour in the eyes of insects. J Comp Physiol A 188:337–348

Stavenga DG (2002b) Reflections on colourful ommatidia of butterfly eyes. J Exp Biol 205:1077–1085

Stavenga DG, Smits RP, Hoenders BJ (1993) Simple exponential functions describing the absorbance bands of visual pigment spectra. Vision Res 33:1011–1017

Stavenga DG, Oberwinkler J, Postma M (2000) Modeling primary visual processes in insect photoreceptors. In: Stavenga DG, DeGrip WJ, Pugh ENJ (eds) Molecular mechanisms in visual transduction. Elsevier, Amsterdam, pp 527–574

Stavenga DG, Kimoshita M, Yang E-C, Arikawa K (2001) Retinal regionalization and heterogeneity of butterfly eyes. Naturwissenschaften 88:477–481

White RH, Xu H, Munch T, Bennett RR, Grable EA (2003) The retina of *Manduca sexta*: rhodopsin-expression, the mosaic of green- blue- and UV-sensitive photoreceptors and regional specialization. J Exp Biol (in press)

Yokoyama S, Yokoyama R (2000) Comparative molecular biology of visual pigments. In: Stavenga DG, DeGrip WJ, Pugh ENJ (eds) Molecular mechanisms in visual transduction. Elsevier, Amsterdam, pp 257–296