

# 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*

JUNKO KITAMOTO<sup>1</sup>, KOICHI OZAKI<sup>2</sup> AND KENTARO ARIKAWA<sup>1,3,\*</sup>

<sup>1</sup>Graduate School of Integrated Science, Yokohama City University, 22-2 Seto, Kanazawa-ku, Yokohama 236-0027, Japan, <sup>2</sup>Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka 560-0043, Japan and <sup>3</sup>PRESTO, Japan Science and Technology Corporation, 4-1-8 Honcho, Kawaguchi 332-0012, Japan

\*Author for correspondence (e-mail: arikawa@yokohama-cu.ac.jp)

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## Summary

This paper describes the primary structures of two opsins of short-wavelength-absorbing visual pigments deduced from the mRNA sequences in the retina of the Japanese yellow swallowtail butterfly *Papilio xuthus*. A phylogenetic analysis of the amino acid sequences indicates that one of these visual pigments is of the ultraviolet-absorbing type and that the other is of the blue-absorbing type. We identified the photoreceptor cells that express these mRNAs by histological *in situ* hybridization. The mRNA of the ultraviolet type is expressed in two distinct

photoreceptor types previously identified as ultraviolet and violet receptors, providing the first molecular biological evidence that different types of spectral receptor probably express a visual pigment with an identical amino acid sequence. The mRNA of the blue type is expressed exclusively in cells classified as blue receptors.

Key words: colour, vision, insect, compound eye, ommatidium, retina, photoreceptor, rhodopsin, fluorescence, butterfly, *Papilio xuthus*.

## Introduction

The compound eye of the Japanese yellow swallowtail butterfly *Papilio xuthus* is composed of approximately 12000 ommatidia, each containing nine photoreceptor cells, R1–R9 (Fig. 1). Intracellular recordings demonstrated that these cells can be classified into at least five different types of spectral receptor, with wavelengths of maximal absorption in the ultraviolet, violet, blue, green or red wavelength regions (Arikawa et al., 1987). Of the four distal photoreceptor cells, R1–R4, which contribute their microvilli to the rhabdom in the distal two-thirds of the ommatidium, R1 and R2 are ultraviolet, violet or blue receptors; R3 and R4 are always green receptors (Bandai et al., 1992). The R5–R8 cells, which contribute their microvilli in the proximal one-third of the rhabdom, are either green or red receptors, and R9, the basal photoreceptor, which adds some microvilli to the rhabdom immediately distal to the basement membrane, is also either a green or a red receptor (Arikawa and Uchiyama, 1996).

The ommatidia of *Papilio xuthus* are clearly spectrally heterogeneous, so how are the ommatidia different, and how are the different ommatidia arranged in the compound eye? An attractive strategy for determining the cellular organization of ommatidia is to clone the mRNAs of the visual pigment opsins and to localize them in the retina by histological *in situ* hybridization. Accordingly, we conducted a molecular

biological study that yielded the mRNAs encoding the opsins of three long-wavelength-absorbing visual pigments and localized their expression in the retina (Kitamoto et al., 1998).

In addition to these three pigment mRNAs, we have now identified mRNAs encoding opsins for an ultraviolet-absorbing and a blue-absorbing visual pigment in the *Papilio xuthus* retina. Combining fluorescence microscopy, electrophysiology and *in situ* hybridization, we found that both ultraviolet and violet receptors express the mRNA of the ultraviolet-absorbing opsin. This lends molecular biological support to our previous hypothesis that the abnormally narrow sensitivity spectrum of violet receptors results from an ultraviolet-absorbing visual pigment that is spectrally filtered by an ultraviolet-absorbing screening pigment located in the same ommatidium (Arikawa et al., 1999b).

## Materials and methods

### Animals

The Japanese yellow swallowtail butterfly *Papilio xuthus* L. was reared in the laboratory. The larvae, hatched from eggs laid by females caught in Yokohama, Japan, were fed on fresh citrus leaves at 25 °C under a 16 h:8 h light:dark photoperiod. Adults were used within 5 days of emergence.

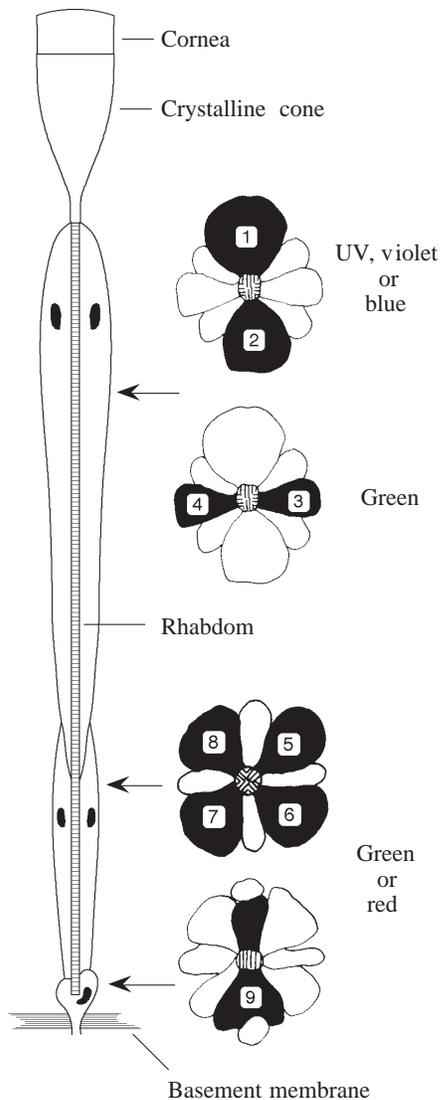


Fig. 1. Schematic drawing of a *Papilio xuthus* ommatidium, which is of the tiered type. 1–4, distal photoreceptors R1–R4; 5–8, proximal photoreceptors R5–R8; 9, basal photoreceptor R9. UV, ultraviolet.

#### Molecular cloning

The method of molecular cloning of *Papilio xuthus* opsins was as described previously (Kitamoto et al., 1998). Briefly, retinal mRNA was first extracted using the QuickPrep Micro mRNA purification kit (Pharmacia) from eyes rapidly frozen in liquid nitrogen. To amplify the cDNAs of short-wavelength-absorbing opsins using reverse transcription/polymerase chain reaction (RT-PCR), we designed a set of degenerate primers based on amino acid sequences conserved in short-wavelength-absorbing opsins of the honeybee *Apis mellifera* (Townson et al., 1998), the locust *Schistocerca gregaria* (Towner et al., 1997), the hawkmoth *Manduca sexta* (Chase et al., 1997) and the tiger swallowtail *Papilio glaucus* (Briscoe, 1998) (see the legend of Fig. 2 for the sequences of the primers). Following RT-PCR using these primers, we identified two DNA fragments with opsin-like sequences. We labelled the fragments with  $^{32}\text{P}$ ,

and used them as probes to screen the *Papilio* retinal cDNA library. The isolated clones did not contain part of the coding region close to the 5' end. This was later determined by 5' rapid amplification of cDNA ends (RACE), and thus the full-length cDNAs were obtained (see Fig. 2).

#### Comparison of amino acid sequences

To compare the amino acid sequences deduced from the cloned cDNAs with opsins described in insects, the sequences were aligned using an alignment program (CLUSTAL W 1.6), and a phylogenetic analysis was then performed using the neighbour-joining method (PHYLIP 3.572), with octopus opsin as an outgroup.

#### In situ hybridization

The compound eyes of *Papilio xuthus* were fixed in 4% paraformaldehyde in 0.1 mol l<sup>-1</sup> sodium phosphate buffer (PB; pH 7.2) for 0.5–2 h at 25 °C and preserved in 30% sucrose in PB at 4 °C overnight. The eyes were then embedded and frozen in Tissue-Tec OCT compound (Miles) and subsequently sectioned at 6–10 µm thickness with a cryostat.

Probes for *in situ* hybridization were designed to hybridize to approximately 200 bases from the mRNAs at the non-coding region downstream of the C terminus. Corresponding cDNA regions were first subcloned into pGEM-3zf(+) vector, and digoxigenin (DIG)-labelled cRNAs were then generated using the DIG-RNA labelling kit (Boehringer Mannheim). The absence of cross-hybridization was confirmed by dot-blot analysis.

Serial sections from a single eye were mounted separately on several slides to label the sections with different probes. One of the slides from a single eye was always labelled with the probe Px-L1, which hybridizes one of the long-wavelength-absorbing opsins we had previously identified, for precise identification of the dorsal and the ventral halves of the eye: Px-L1 is detected only in ventral regions (Kitamoto et al., 1998).

For labelling, sections were treated with hybridization solution containing 0.5 µg ml<sup>-1</sup> of the cRNA probe at 65 °C overnight. After a brief rinse, the sections were incubated in 50% formamide in 2× SSC (1× SSC is 0.15 mol l<sup>-1</sup> NaCl, 0.015 mol l<sup>-1</sup> sodium citrate) at 65 °C for 2 h, and then treated with RNAase (10 µg ml<sup>-1</sup>) at 37 °C for 1 h. The probes were visualized using anti-DIG immunocytochemistry.

#### Fluorescence microscopy

We previously found that some ommatidia of *Papilio xuthus* fluoresce under ultraviolet epi-illumination, both *in vivo* and in sections (Arikawa et al., 1999b; Arikawa and Stavenga, 1997). To correlate the ultraviolet-induced autofluorescence with the results of the *in situ* hybridization, we quickly video-recorded the fluorescence of the frozen sections, because the fluorescence easily bleaches under prolonged ultraviolet epi-illumination. The frozen sections were then processed for *in situ* hybridization and further imaging. The video-recorded images were printed and then compared.

## Results

We identified two novel mRNAs encoding opsins in addition to the mRNAs of the opsins of the three long-wavelength-absorbing visual pigments identified previously (Kitamoto et al., 1998). As we shall explain in detail below, it appeared that the encoded opsins are of the ultraviolet- and blue-absorbing types. We therefore term them Px-UV (*Papilio xuthus*-UV) and Px-B, respectively. For clarity, we here re-term the opsins of the long-wavelength-absorbing visual pigments PxRh1, PxRh2 and PxRh3 (Kitamoto et al., 1998) as Px-L1, Px-L2 and Px-L3 (for *Papilio xuthus* long-wavelength-absorbing types 1–3) respectively.

### Primary structures of two novel opsins

The cDNAs of Px-UV and Px-B have single open reading frames of 1140 bp and 1143 bp, encoding 380 and 381 amino acid residues, respectively (Fig. 2). Both Px-UV and Px-B have amino acid residues conserved in other opsins, such as lysine in the seventh helix for chromophore attachment (Wang et al., 1980) and cysteines for disulphide bond formation (Karnik et al., 1988). The amino acid residue in the third helix corresponding to the Schiff base counter-ion in vertebrate opsins was phenylalanine in Px-UV and tyrosine in Px-B.

Fig. 3 shows the phylogenetic relationships of the insect opsins identified to date, including Px-UV and Px-B. Px-UV falls into the group of the ultraviolet-absorbing types, whereas Px-B is in the group of the blue-absorbing types.

### Histological *in situ* hybridization

The photoreceptor cells expressing Px-UV and Px-B were identified by histological *in situ* hybridization on frozen sections of the eye. Histological *in situ* hybridization demonstrates only that the photoreceptor cells contain mRNAs,

not Px-UV and Px-B themselves, although these mRNAs are likely to be translated into functional proteins.

Fig. 4A,C shows oblique sections labelled with the Px-UV and the Px-B probe, respectively. The labelling was found exclusively in the distal two-thirds (Fig. 4A) or the middle portion (Fig. 4C) of the photoreceptor layer. The distal photoreceptors bear their rhabdomeral microvilli in the distal two-thirds of the rhabdom (Arikawa and Uchiyama, 1996). The densely labelled portion corresponds to the region immediately proximal to their nuclei. At any rate, the proximal photoreceptors (R5–R8), which contribute their microvilli only to the proximal one-third of the rhabdom, are not labelled with these probes.

The other five sections shown in Fig. 4 were obtained from the same region of an eye: the white polygon indicates the corresponding area on each section. Fig. 4E is the ommatidial autofluorescence photographed immediately after the section was cut in the cryostat. Fig. 4B,D,F,G were labelled with probes that hybridize specifically to the mRNAs of Px-UV, Px-B, Px-L2 and Px-L3, respectively.

In the distal tier, the mRNAs of Px-UV and Px-B were found only in R1 and R2 photoreceptors: the other photoreceptors, R3–R9, were not labelled. In Fig. 4B,D, there are three types of ommatidia in terms of the mRNA they contain in the R1 and R2 photoreceptors. Type 1 ommatidia have Px-UV mRNA in R1 and Px-B mRNA in R2 (right-hand red circle) or *vice versa* (left-hand red circle). Type 2 has Px-UV mRNA in both R1 and R2 (pink circle). This type corresponds to those that emit strong fluorescence under ultraviolet epi-illumination (Fig. 4E). The other ommatidia, which contain the Px-B mRNA in both R1 and R2, are type 3 (yellow circle). By comparing Fig. 4B and Fig. 4D, it is clear that all R1 and R2 photoreceptors are labelled with either one of these probes and

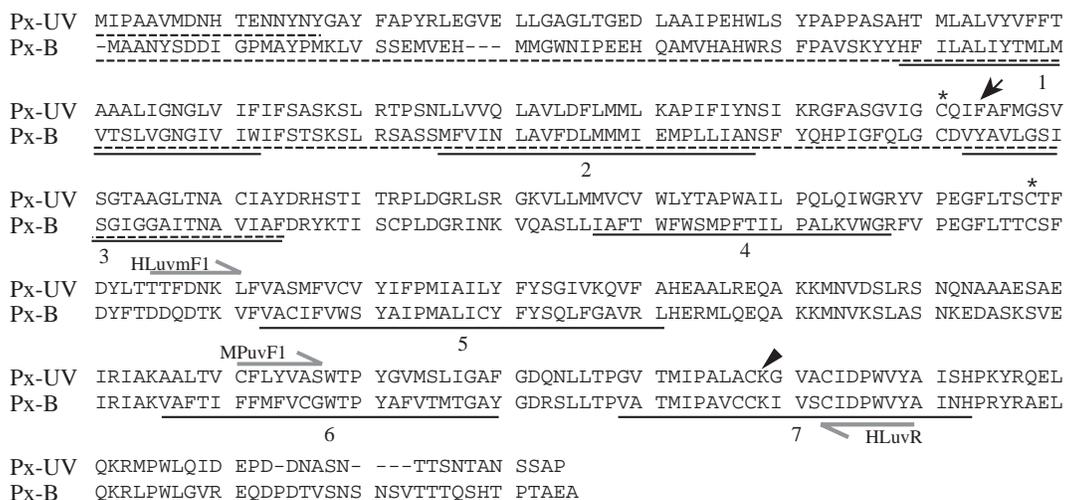
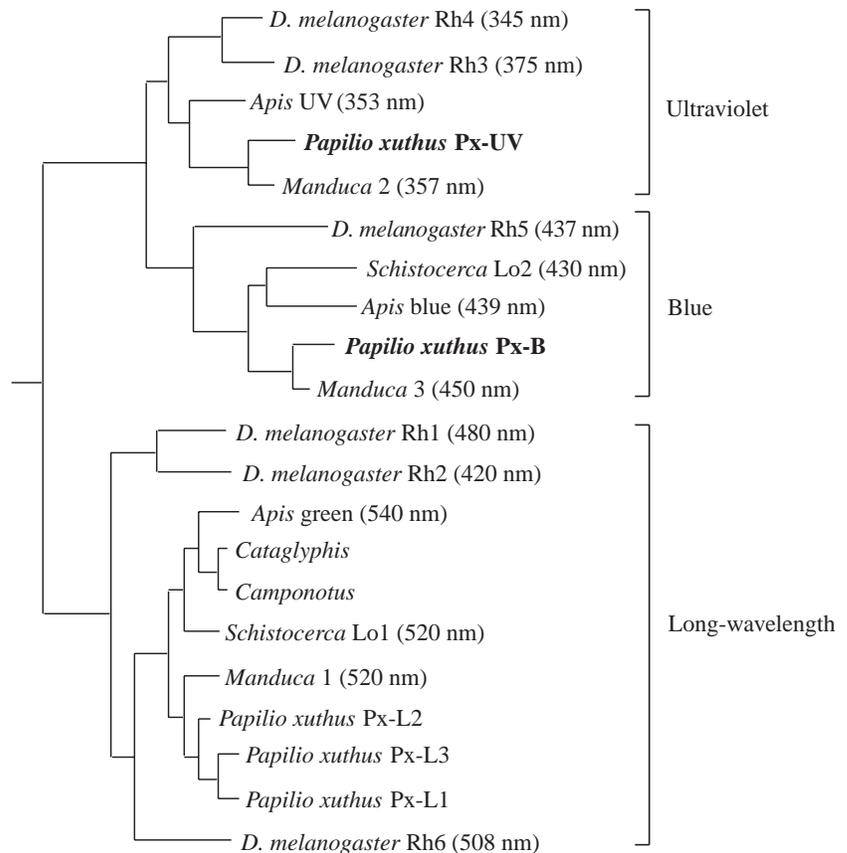


Fig. 2. Deduced amino acid sequences of opsins Px-UV and Px-B. Hatched half-arrows indicate regions corresponding to primer attachment: the primer sequences are GCGAATTCGAYGAYGARGAYACNAARGT (HLuvmF1), GCGAATTCGYTTYTYTNTTYGTNGC (MPuvF1) and CGGGATCCTANAYCCANGGRTCDATRCA (HLuvR). Broken underlining indicates the regions determined by 5' RACE. Large arrow, position corresponding to the Schiff base counter-ion in vertebrate opsins; arrowhead, lysine for chromophore attachment; asterisks, cysteines for disulphide bond formation. Data accession numbers for Px-UV and Px-B are AB028218 and AB028217, respectively.

Fig. 3. Phylogenetic relationship of insect visual pigment opsins calculated using the neighbour-joining method with octopus opsin as the outgroup. Wavelengths indicate the wavelength of maximal absorption ( $\lambda_{\max}$ ) of the absorption spectrum of these visual pigments estimated by intracellular electrophysiology of animals *in situ* [*Schistocerca gregaria*, Bennett et al. (1967); *Manduca sexta*, White et al. (1983); *Apis mellifera*, *Apis* green, Menzel et al. (1986)] or by electroretinogram measurement of transgenic *Drosophila melanogaster* in which the corresponding opsins are expressed [*Apis* UV and *Apis* blue, Townson et al. (1998); *Drosophila* Rh1, Zuker et al. (1988); *Drosophila* Rh2, Feiler et al. (1988); *Drosophila* Rh3 and Rh4, Feiler et al. (1992); *Drosophila* Rh5 and Rh6, Salcedo et al. (1999)]. Data accession numbers: *Apis* UV, AF004169; *Apis* blue, AF004168; *Apis* green, U26026; *Camponotus*, U32502; *Cataglyphis*, U32501; *D. melanogaster* Rh1, A90864; *D. melanogaster* Rh2, A24058; *D. melanogaster* Rh3, Y00043; *D. melanogaster* Rh4, M17730; *D. melanogaster* Rh5, U80667; *D. melanogaster* Rh6, Z86118; *Manduca* 1, L78080; *Manduca* 2, L78080; *Manduca* 3, AD001674; *Papilio xuthus* Px-L1 (PxRh1), AB007423; *Papilio xuthus* Px-L2 (PxRh2), AB007424; *Papilio xuthus* Px-L3 (PxRh3), AB007425; *Schistocerca* Lo1, X80071; *Schistocerca* Lo2, X80072.



also that no R1 and R2 photoreceptors are double-labelled with them.

Fig. 4F,G are adjacent sections through the proximal tier. These sections were labelled with probes detecting Px-L2 (Fig. 4F) and Px-L3 (Fig. 4G) mRNAs, which have been shown to be expressed in the green and red receptors (Kitamoto et al., 1998). In Fig. 4F, the labelling density of the R5–R8 proximal photoreceptors is evidently variable between ommatidia: no labelling, weak labelling and strong labelling all occurred (Arikawa, 1999; Kitamoto et al., 1998). Apparently, these variations correspond to three ommatidial types; i.e. no labelling for type 1; weak labelling for type 2 and strong labelling for type 3. The R5–R8 photoreceptors of type 2 ommatidia co-express Px-L3 mRNA (Fig. 4G).

Fig. 4H shows the organization of three types of ommatidia in the area encircled by the polygon. Of the 32 ommatidia in this particular tissue, 18 are of type 1 (56.3%), six are of type 2 (18.7%) and eight are of type 3 (25.0%).

## Discussion

### *Px-UV and Px-B opsins*

In this paper, we describe two novel mRNAs encoding visual pigment opsins identified in the compound eye of *Papilio xuthus*. Histological *in situ* hybridization showed that these opsin mRNAs are expressed exclusively in the R1 or R2 photoreceptors in the distal tier of the ommatidia. Previously,

we found that R1 and R2 are ultraviolet, violet or blue receptors (Bandai et al., 1992) and, hence, we conclude that the opsins are short-wavelength-absorbing visual pigments.

We have compared the amino acid sequences of the novel opsins deduced from the cDNA sequences with those of other insect opsins (Fig. 3). The phylogenetic analysis indicates that one of the new opsins is a member of the group including *Manduca* 2 (Chase et al., 1997), *Apis* UV (Townson et al., 1998), *Drosophila* Rh3 (Zuker et al., 1987) and *Drosophila* Rh4 (Montell et al., 1987), all presumed to be of the ultraviolet-absorbing type. The other appears in the group of blue-absorbing opsins, including *Manduca* 3 (Chase et al., 1997), *Apis* blue (Townson et al., 1998) and *Drosophila* Rh5 (Chou et al., 1996). Together with our *in situ* hybridization results, discussed below, this indicates that these novel opsins are of the ultraviolet- and blue-absorbing class, respectively. We therefore termed these opsins as Px-UV and Px-B. Convincing proof of the spectral absorbance characteristics of the visual pigments could be provided by expression experiments, but these have not yet been completed.

### *Violet and ultraviolet receptors share Px-UV mRNA*

The violet receptor in the retina of *Papilio xuthus* exhibits an abnormally narrow sensitivity spectrum (Arikawa et al., 1987). The physiological basis of the narrow sensitivity spectrum has only recently been resolved. In the ventral half of the eye, some ommatidia emit strong, whitish fluorescence

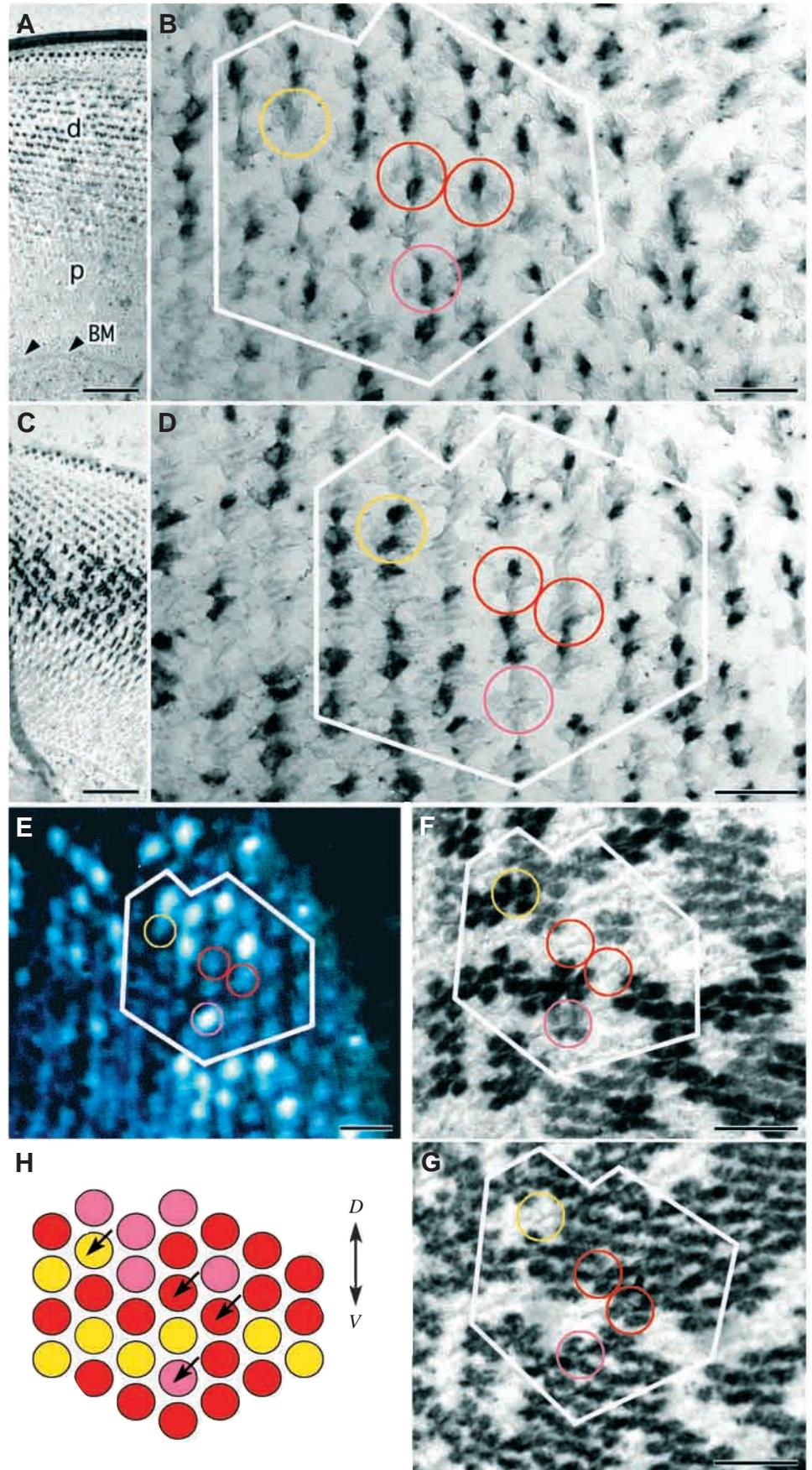


Fig. 4. *In situ* hybridization in serial sections of the same region of the eye (not A and C). Identical ommatidia are marked by coloured symbols. (A) Oblique section labelled with the Px-UV probe. The distal tier (d) is labelled, whereas the proximal tier (p) is free of labelling. BM, basement membrane. (B) Transverse section through the distal tier labelled with the Px-UV probe. (C) Oblique section labelled with the Px-B probe. Only the distal tier is labelled. (D) Transverse section through the distal tier labelled with the Px-B probe. (E) Transverse section through the most distal part of the retina; ultraviolet-induced autofluorescence. (F) Transverse section of the proximal tier labelled with the Px-L2 probe. (G) Transverse section of the proximal tier labelled with the Px-L3 probe. (H) Three types of ommatidia are identified: red, type 1; pink, type 2; yellow, type 3. These colours correspond to the transmission of each ommatidial type in eye-slice preparations (see Arikawa and Stavenga, 1997). Arrows indicate the ommatidia indicated by coloured circles in B and D-G. See also Tables 1 and 2. D, dorsal; V, ventral; Scale bars, 100  $\mu$ m (A,C); 60  $\mu$ m (B,D-G).

when illuminated by ultraviolet light (Arikawa and Stavenga, 1997). The fluorescing material, probably 3-hydroxyretinol (Arikawa et al., 1999b), is concentrated in the distal one-fifth of the ommatidia (Fig. 4E; see Fig. 2 in Arikawa et al., 1999b). This localization suggested a specific function of the 3-hydroxyretinol; namely, that it acts as an ultraviolet-absorbing spectral filter for the photoreceptors underneath. We therefore constructed an optical model taking into account the detailed anatomy of *Papilio xuthus* ommatidia. When the R1 and R2 photoreceptors in the fluorescing ommatidia contain an ultraviolet-absorbing visual pigment, the model predicts that the sensitivity spectrum of the photoreceptors will become distinctly narrower than the absorption spectrum of the ultraviolet visual pigment and, furthermore, that the peak will be shifted to the violet, just as is found for the sensitivity spectrum of the violet receptor. The appropriateness of the ultraviolet-filter hypothesis was strengthened by further electrophysiological experiments: violet receptors were always found in the fluorescing ommatidia, whereas ultraviolet receptors were found only in the non-fluorescing ommatidia (Arikawa et al., 1999b).

In the simplest version of the above hypothesis, we assumed that both the violet and the ultraviolet receptors have the same ultraviolet-absorbing visual pigment. Almost to our surprise, the present *in situ* hybridization results strongly suggest that this is indeed the case. One could argue that the Px-UV probe might have detected multiple mRNAs encoding ultraviolet visual pigments with similar sequences each separately expressed in the ultraviolet receptors and the violet receptors. However, our probe was designed to hybridize to the non-coding region downstream from the C terminus, so that there is very little likelihood that the Px-UV probe hybridizes to two different mRNAs in this position. As is clearly demonstrated in Fig. 4, the fluorescing ommatidia contain Px-UV in both R1 and R2.

Px-UV is also expressed in the R1 or R2 photoreceptors in some other, non-fluorescing ommatidia. The Px-UV-

Table 1. Frequency of three types of ommatidia in the ventral and dorsal regions of the compound eye of *Papilio xuthus*

Type (opsins in R1/R2)	Ventral (%)	Dorsal (%)
Type 1 (Px-UV/Px-B)	41.4 (1535)	40.7 (177)
Type 2 (Px-UV/Px-UV)	31.9 (1182)	8.7 (38)
Type 3 (Px-B/Px-B)	26.7 (988)	50.6 (220)
Total	100 (3705)	100 (435)

Numbers of ommatidia observed are given in parentheses.

containing R1 and R2 photoreceptors then behave as ultraviolet receptors.

The above discussion has focused only to the ventral half of the eye, where the type 2 ommatidia are always of the fluorescing type. Ommatidial fluorescence is obscured in the dorsal half of the eye, probably because of corneal fluorescence (Arikawa et al., 1999b). Although making only minor contribution, type 2 ommatidia are present in the dorsal half of the eye (Table 1). The R1 and R2 photoreceptors could therefore be ultraviolet receptors, but this has yet to be demonstrated electrophysiologically.

#### Blue receptors

The R1 and R2 photoreceptors are ultraviolet, violet or blue receptors (Bandai et al., 1992). These photoreceptors are labelled with either the Px-UV or the Px-B probe. As discussed above, the ultraviolet and violet receptors contain Px-UV mRNA. Hence, the R1 and R2 photoreceptors labelled with the Px-B probe must be blue receptors.

#### An identical visual pigment in different spectral receptors

The present results suggest that a visual pigment of identical amino acid sequence exists in the different spectral receptors that were identified electrophysiologically. The existence of identical visual pigments in different spectral receptors has

Table 2. Three types of ommatidia in the compound eye of *Papilio xuthus*

Fluorescence: Pigmentation:	Type 1		Type 2		Type 3	
	No Red	Yes Red	Yes Red	No Yellow	No Yellow	Yes Yellow
	<i>S</i> (λ)	Opsin	<i>S</i> (λ)	Opsin	<i>S</i> (λ)	Opsin
R1	UV*	Px-UV*	V	Px-UV	B	Px-B
R2	B*	Px-B*	V	Px-UV	B	Px-B
R3–R4	DG	Px-L1+L2	SG	Px-L1+L2	DG	Px-L1+L2
R5–R8	R	Px-L3	A	Px-L2+L3	DG	Px-L2
R9	?	?	?	?	?	?

Spectral sensitivity, *S*(λ), and the visual pigment opsins expressed in photoreceptors in the ventral half of the eye are shown.

UV, ultraviolet; V, violet; B, blue; DG, double-peaked green; SG, single-peaked green; R, red; A, abnormally broad.

Photoreceptors R3 and R4 of all ommatidia co-express two visual pigments.

For details of *S*(λ) of UV, V, DG and SG pigments, see Bandai et al. (1992) and Arikawa et al. (1999b).

For *S*(λ) of R and A pigments, see Arikawa et al. (1999c) and Arikawa et al. (1999a), respectively.

For visual pigments, see Kitamoto et al. (1998).

For Type 1, the spectral type of R1 and R2 may be interchanged (asterisks).

been suggested previously in birds. In chickens, for example, the visual pigment absorbing at 571 nm appeared to be contained both in the principal member of the double cone containing greenish oil droplet and in the single cone containing reddish oil droplet. Because of the difference in the colour of the oil droplets, which act as spectral filters, the predicted absorption spectra for the outer segments of the double cone principal member and the single cone are distinctly different: the spectrum of the single cone is narrow and the peak is shifted to 600 nm (Bowmaker and Knowles, 1977; Okano et al., 1995).

However, in birds, electrophysiological measurements of the sensitivity spectrum of each photoreceptor are lacking. In this regard, the present case of *Papilio xuthus* fills the gap, although the presence of the identical visual pigment molecule itself in the ultraviolet and violet receptors has yet to be demonstrated by, for example, immunocytochemistry using specific antibodies.

#### *Cellular organization of three types of Papilio xuthus ommatidium*

We have suggested that there are at least three types of spectrally distinct ommatidia in the eye of *Papilio xuthus* on the basis of the distribution of colour filters (Arikawa and Stavenga, 1997) and of the mRNAs encoding long-wavelength-absorbing opsins (Kitamoto et al., 1998). Here, we again characterized three types of ommatidia on the basis of the mRNA encoding the short-wavelength-absorbing opsins they contain. Taking all the information together, we now have an almost complete picture of the cellular organization of the ommatidia of *Papilio xuthus* (Table 2).

We showed previously that both Px-L1 and Px-L2 mRNAs are expressed in green receptors, whereas Px-L3 mRNA is expressed in red receptors (Kitamoto et al., 1998). Moreover, an electrophysiological study revealed that green-sensitive receptors R5–R8 are always found in yellow-pigmented ommatidia and that red-sensitive receptors R5–R8 are found exclusively in red-pigmented ommatidia (Arikawa et al., 1999c). Type 1 ommatidia with Px-L3 mRNA in R5–R8 photoreceptors (i.e. red receptors) must have red pigmentation. Similarly, type 3 ommatidia whose R5–R8 photoreceptors contain Px-L2 mRNA are yellow-pigmented. R3 and R4 photoreceptors in type 1 and type 3 ommatidia are normal green receptors with a secondary peak in the ultraviolet wavelength region (double-peaked green), which double-express Px-L1 and Px-L2 mRNAs.

We also showed that some R5–R8 photoreceptors are double-labelled by the Px-L2 and Px-L3 probes (Fig. 4E,G; see also Kitamoto et al., 1998). Clearly, the ommatidia containing the double-labelled R5–R8 photoreceptors are of type 2 (Fig. 4). Another characteristic of type 2 ommatidia is ultraviolet-induced fluorescence (Arikawa et al., 1999b). The fluorescing ommatidia belong to a subset of red-pigmented ommatidia (Arikawa and Stavenga, 1997). Interestingly, the sensitivity spectra of the R5–R8 photoreceptors in the fluorescing ommatidia is abnormally broad (i.e. the half-

bandwidth is approximately 270 nm), suggesting different absorption spectra for the two expressed visual pigments. The anomalous cells are called A cells (Arikawa et al., 1999a).

The properties of R9 photoreceptors need further elucidation.

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