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Review

Reconstructing the ancestral butterfly eye: focus on the opsins

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Summary

The eyes of butterflies are remarkable, because they are nearly as diverse as the colors of wings. Much of eye diversity can be traced to alterations in the number, spectral properties and spatial distribution of the visual pigments. Visual pigments are light-sensitive molecules composed of an opsin protein and a chromophore. Most butterflies have eyes that contain visual pigments with a wavelength of peak absorbance, λ_{max}, in the ultraviolet (UV, 300–400 nm), blue (B, 400–500 nm) and long wavelength (LW, 500–600 nm) part of the visible light spectrum, respectively, encoded by distinct UV, B and LW opsin genes. In the compound eye of butterflies, each individual ommatidium is composed of nine photoreceptor cells (R1–9) that generally express only one opsin mRNA per cell, although in some butterfly eyes there are ommatidial subtypes in which two opsins are co-expressed in the same photoreceptor cell. Based on a phylogenetic analysis of opsin cDNAs from the five butterfly families, Papilionidae, Pieridae, Nymphalidae, Lycaenidae and Riodinidae, and comparative analysis of opsin gene expression patterns from four of the five families, I propose a model for the patterning of the ancestral butterfly eye that is most closely aligned with the nymphalid eye. The R1 and R2 cells of the main retina expressed UV–UV-, UV–B- or B–B-absorbing visual pigments while the R3–9 cells expressed a LW-absorbing visual pigment. Visual systems of existing butterflies then underwent an adaptive expansion based on lineage-specific B and LW opsin gene multiplications and on alterations in the spatial expression of opsins within the eye. Understanding the molecular sophistication of butterfly eye complexity is a challenge that, if met, has broad biological implications.

Key words: eye evolution, color vision, photoreceptor, rhodopsin, visual pigment, opsin, sexual dimorphism.

Introduction

The butterfly eye is a marvel of evolution. Butterfly vision, like that of other insects, is based on three major classes of photoreceptor, with peak sensitivity (λ_{max}) in the ultraviolet (UV, 300–400 nm), blue (B, 400–500) and long wavelength (LW, 500–600 nm) part of the light spectrum. At the molecular level, these visual pigments are composed of a retinal-based chromophore (e.g. 11-*cis*-3-hydroxyretinal) (Smith and Goldsmith, 1990) attached by a Schiff-base linkage to an opsin protein. The spectral tuning of the visual pigment wavelength of peak absorbance, λ_{max}, is achieved through the interaction of the chromophore with critical amino acid residues within the opsin. Changes in the polarity of amino acids in the chromophore-binding pocket of opsins, for example, affect the distribution of electrons in the π-electron system of the chromophore, producing a diversity of λ_{max} values (Honig et al., 1976). In the case of the butterfly visual pigments, the three major spectral classes are encoded by ancient duplications, which produced distinct UV, B and LW opsin genes.

Unlike those of bees, the eyes of butterflies are anatomically and physiologically diverse (Peitsch et al., 1992; Briscoe and Chittka, 2001; Stavenga and Arikawa, 2006; Frentiu et al., 2007b; Frentiu et al., 2007a). This is due in part to lineage-specific opsin gene duplications, as well as to changes in the kind and distribution of lateral filtering pigments (Arikawa and Stavenga, 1997; Stavenga, 2002).

To understand this diversity, this review focuses on the eyes of four species of butterfly for which we have the most complete information, *Danaus plexippus* Linnaeus, *Lycaena rubidus* Behr, *Pieris rapae crucivora* Boisduval and *Papilio xuthus* Linnaeus, and whose eyes serve as prototypes for the butterfly families Nymphalidae, Lycaenidae, Pieridae and Papilionidae, respectively. Although the importance of non-opsin-based filtering pigments for modifying photoreceptor sensitivity to light is mentioned, it is not yet clear whether the filtering pigments in different families are homologous or not; therefore I have intentionally spotlighted the evolution of the opsins themselves because of the homologous relationship between opsin protein sequences between butterfly families and the spectral range of vision. By analyzing the physiologically determined photopigment absorbance spectra and the phylogenetic relationship of opsin gene sequences, overlaid with a character map of opsin expression patterns, I propose a novel model of the evolutionary events leading from an ancestral butterfly eye to the radiation of eye types observed today. And with an eye to the future, I emphasize a few fertile areas for further investigation.

Anatomy of the butterfly eye

Anatomically, the basic unit of the butterfly compound eye is the ommatidium. An ommatidium is composed of nine photoreceptor cells (R1–9) along with primary and secondary pigment cells. An ommatidium from the simplest eye, that of nymphalid butterflies, consists of two tiers composed, respectively, of eight photoreceptor cells (R1–8) that are elongated, and a tiny R9 cell that sits just above the basement membrane (Fig. 1A). Light passing through the cornea is focused by the crystalline cone onto the rhabdom. The fused

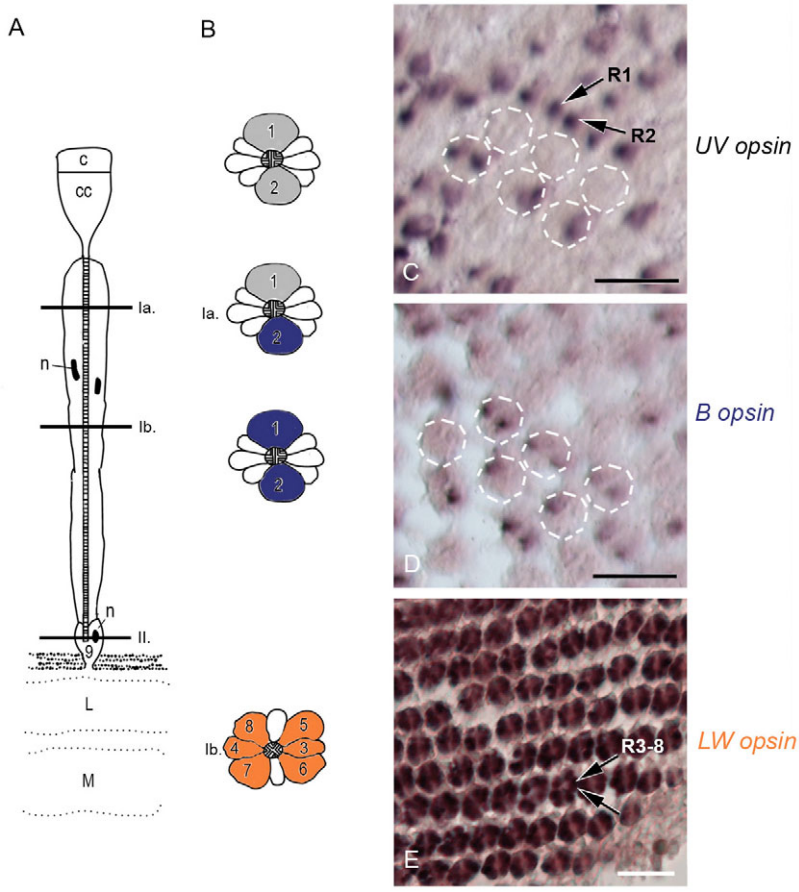

Fig. 1. Diagram of an ommatidium and pattern of ultraviolet (UV), blue (B) and long wavelength (LW) opsin mRNA in the main retina of the monarch, *Danaus plexippus* (modified from Sauman et al., 2005). (A) Longitudinal view of an ommatidium. In nymphalids, the photoreceptor cell bodies that contribute to the fused rhabdom are organized into two tiers, composed of the R1–8 cells (tier I) and the R9 cell (tier II). Thick black lines, Ia. and Ib., indicate the approximate level from which the tangential sections of the R1–8 cells shown to the right were taken. c, cornea; cc, crystalline cone; n, photoreceptor cell nucleus; L, lamina; M, medulla. (B) Tangential views of ommatidial subtypes. Gray, purple and orange indicate the identity of the photoreceptor cells in which specific opsin mRNA expression is shown in the panels to the right. (C) Tangential section showing specific labeling of R1 and R2 cells with a digoxigenin-labeled antisense UV opsin riboprobe. Dashed circles indicate boundaries of identical individual ommatidia to those probed for B opsin mRNA in D. Scale bar, 25 μm. (D) Tangential section showing B opsin mRNA expression in an adjacent section to that shown in C. Dashed circles indicate identical ommatidia. Three subtypes of ommatidia are evident with respect to opsin expression in R1 and R2 photoreceptor cells: B–B, B–UV, UV–UV. Scale bar, 25 μm. (E) Tangential section showing LW opsin mRNA expression in all R3–8 cells (arrows). Scale bar, 25 μm.

rhabdom is composed of nine rhabdomeres, the microvillous membranes protruded by the individual photoreceptor cells, in which the visual pigments are embedded.

UV and B opsin mRNA expression define three ommatidial subtypes in the main retina

The ommatidia of the main retina of the nymphalid eye are divided into three subtypes with respect to opsin mRNA expression, with R1 and R2 photoreceptor cells expressing UV–UV, UV–B or B–B, while the outer R3–8 cells express the LW opsin (Fig. 1B) (Briscoe et al., 2003). The monarch butterfly, *Danaus plexippus*, is a nymphalid butterfly whose eye typifies this pattern of opsin mRNA expression (Fig. 1C–E) (Sauman et al., 2005). The identity of the opsin expressed in the R9 cell of monarchs is unknown, but is likely to be the same as in another nymphalid *Vanessa cardui*, in which R9 expresses the LW opsin (Briscoe et al., 2003).

In addition to the ommatidia of the main retina, butterfly eyes also contain a dorsal rim area (DRA), like that found in other insects (Labhart and Meyer, 1999). In monarchs, the DRA R1–8 cells express the UV opsin exclusively (Sauman et al., 2005); these ommatidia contain rhabdoms whose microvilli are anatomically specialized to detect polarized skylight (Reppert et al., 2004). This monochromatic pattern of opsin expression in the DRA is important for efficient perception of polarized light, by avoiding interference with color information (Labhart and Meyer, 1999).

Spectral diversity of butterfly visual pigments

The wavelength of peak absorbance, λ_{max}, of visual pigments can be directly measured using epi-microspectrophotometry, or derived from the spectral sensitivity curves of intracellular recordings of individual photoreceptor cells. In both cases, the experimental data are matched to an idealized rhodopsin template (Stavenga et al., 1993; Palacios et al., 1996), and a computational model is then applied to find the best estimate of λ_{max}.

Based on such measurements, the spectral diversity of visual pigments in the eyes of butterflies is striking. As already alluded to, the eye of the monarch contains the smallest number of butterfly photopigments (P) with one UV (λ_{max}=340 nm or P340), one B (P435) and one LW (P545) (Stalleicken et al., 2006; Frentiu et al., 2007a) (Fig. 2A). The lycaenid *Lycaena rubidus* eye contains four photopigments: one UV (P360), two B (P437 and P500) and one LW (P568) (Bernard and Remington, 1991) (Fig. 2B). The papilionid *Papilio xuthus* eye contains the largest number of butterfly photopigments so far described, with one UV (P360), one B (P460) and three LW (P515, P530 and P575) (Arikawa, 2003) (Fig. 2C). The eye of the pierid *Pieris rapae* contains four photopigments: one UV (P360), two B (P425 and P453) and one LW (P563) (Qiu and Arikawa, 2003a; Qiu and Arikawa, 2003b) (Fig. 2D).

Implications of B and LW opsin gene duplications in butterfly eye evolution

The opsin cDNAs encoding each of the visual pigments have been isolated (Kitamoto et al., 1998; Kitamoto et al., 2000; Wakakuwa et al., 2004; Arikawa et al., 2005; Sauman et al., 2005; Sison-Mangus et al., 2006; Frentiu et al., 2007a), and with these data a gene tree has been constructed for each of the three spectral classes of opsin: UV, B and LW (Fig. 3A–C, respectively). Species from all five butterfly families, Papilionidae (*Papilio xuthus* and *P. glaucus*

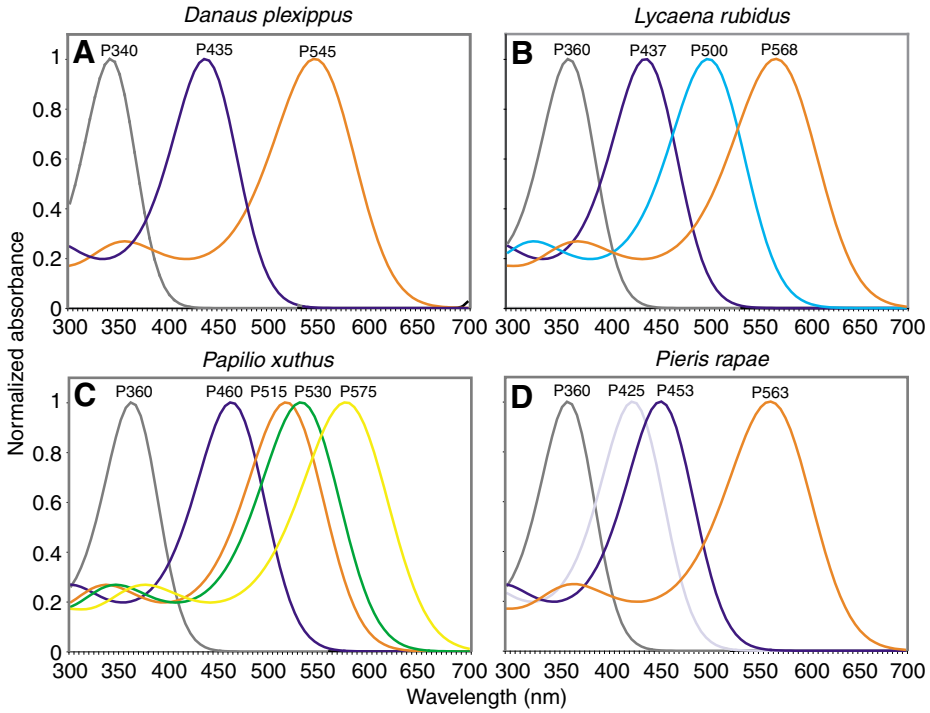

Fig. 2. Normalized absorbance spectra of butterfly visual pigments based upon the Bernard template (see Palacios et al., 1996) and wavelengths of peak absorbance (λ_{max}) estimated from either microspectrophotometry or intracellular recordings. (A) The compound eye of the nymphalid *Danaus plexippus* contains three photopigments (P) with λ_{max} values corresponding to 340 nm (gray), 435 nm (purple) and 545 nm (orange), respectively. Estimates of the λ_{max} values of the UV (P340) and B (P435) photopigments are from electrophysiological recordings (Stalleicken et al., 2006) and for the LW (P545) photopigment, from microspectrophotometric measurements (Frentiu et al., 2007a). (B) The eye of the lycaenid *Lycaena rubidus* contains four photopigments: P360 (gray), P437 (purple), P500 (blue), P568 (orange). Estimates of the λ_{max} values are from microspectrophotometric measurements (Bernard and Remington, 1991). (C) The eye of the papilionid *Papilio xuthus* contains five photopigments: P360, P460, P515, P530 and P575. Estimates of the λ_{max} values are from the intracellular recordings (Arikawa, 2003). (D) The eye of the pierid *Pieris rapae* contains four photopigments: P360 (gray), P425 (violet), P453 (purple) and P563 (orange). Estimates of the λ_{max} values are from intracellular recordings (Qiu and Arikawa, 2003a; Qiu and Arikawa, 2003b) (see also Wakakuwa et al., 2004; Arikawa et al., 2005).

Linnaeus), Pieridae (*Pieris rapae*), Nymphalidae (*Danaus plexippus*), Lycaenidae (*Lycaena rubidus*) and Riodinidae (*Apodemia mormo* Felder and Felder) have been included to illustrate the astonishing fact that independent duplications of either the B or LW opsin have occurred in each family, rendering the eyes of butterflies the most diverse yet characterized among insects.

For each of the butterfly family examples, only one UV opsin gene has been isolated, including from the riodinid *Apodemia mormo*, presented here for the first time (Fig. 3A). By contrast, duplicate B opsin genes have been isolated from *P. rapae*, encoding blue-absorbing (P453) and violet (V)-absorbing (P425) photopigments (Arikawa et al., 2005), and from *L. rubidus*, encoding blue-absorbing (P437) and blue–green-absorbing (P500) photopigments (Sison-Mangus et al., 2006). The bootstrap values of the relevant nodes and branching order of the tree, which nicely matches the current phylogeny of butterfly families based on independent molecular and morphological markers (see below) (Wahlberg et al., 2005), indicate that the duplication of the blue opsin gene in pierids and in lycaenids occurred independently.

The situation for the LW opsins, with respect to the frequency of gene duplication, is more complex. The eyes of pierids, nymphalids and lycaenids express only one LW opsin gene encoding photopigments of P563, P545 and P568, respectively (Wakakuwa et al., 2004; Sauman et al., 2005; Sison-Mangus et al., 2006), while the papilionid eye expresses three (P515, P530 and P575) (Kitamoto et al., 1998) and the riodinid eye expresses two LW opsin genes (P505 and P600) (Frentiu et al., 2007a) (Fig. 3C). To add more complexity, at least one additional near full-length LW opsin cDNA, *PglRh4*, for a total of four LW opsin genes, has been isolated from head tissue of *Papilio glaucus* (Briscoe, 1998; Briscoe, 2000) – an opsin that is not apparently expressed in the eye (A.D.B., unpublished data) (Lampel et al., 2005). The presence of two LW opsin genes in the genome of the silkmoth *Bombyx mori*, and the apparent close homology of the sphingid moth *Manduca sexta* opsin gene *MANOP1* with one of them (Fig. 3C), suggests that *M. sexta* too may contain a second LW opsin, which is a homolog of the silkmoth larval 'brain opsin' gene *Boceropsin* (Shimizu et al., 2001).

The fate of duplicate genes is typically classified into one of two categories: subfunctionalization ('division of labor'), in which one or both of the paralogs acquires a more restricted expression pattern and/or biological activity than the common ancestral gene, and neofunctionalization, in which the paralogs are free to acquire new expression patterns and/or functions (Force et al., 1999). Opsin duplication in butterflies can fall into either category (Briscoe, 2001). Importantly, duplication of butterfly opsin genes has led to the evolution of novel λ_{max} values that ultimately impact on behavior (Kelber, 1999; Kelber and Pfaff, 1999; Kinoshita et al., 1999).

Subfunctionalization of B opsins in *Pieris*

In *Pieris rapae*, LW opsin expression is identical to that of the nymphalid eye in that its single LW opsin transcript is expressed in R3–8 (Wakakuwa et al., 2004). In addition, the expression of the

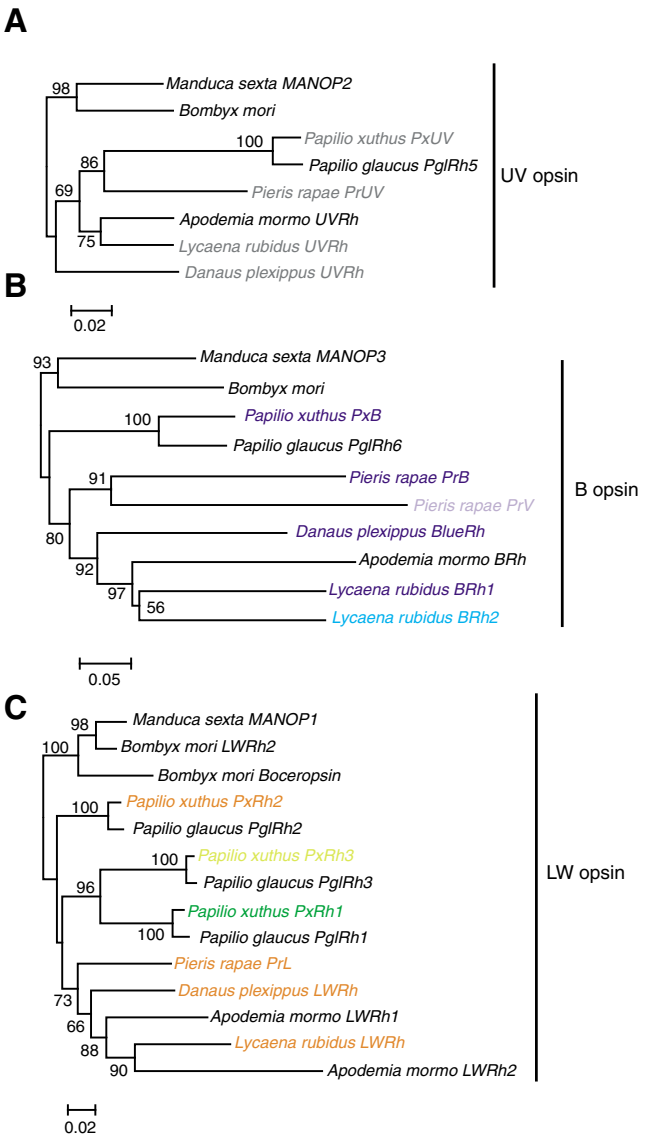

Fig. 3. Phylogenies of the UV, B and LW opsin genes of each of the five butterfly families including genes corresponding to the visual pigments shown in Fig. 2. The neighbor-joining method using 1st+2nd nucleotide positions and the Tamura-Nei model of evolution was used. Numbers represent the percentage of trees in which a particular node was recovered out of 500 replicates. The moths *Manduca sexta* (Sphingidae) and *Bombyx mori* (Bombycidae) are used as outgroups. (A) UV opsin gene tree. Opsin genes encoding the UV-absorbing visual pigments in Fig. 2 are indicated by bold gray. In each of the butterfly species shown, the UV visual pigment is encoded by a single-copy gene. The full-length UV opsin cDNA of *A. mormo* is newly presented in this study (GenBank accession no. AY587905). (B) B opsin gene tree. Opsin genes encoding the B-absorbing visual pigments in Fig. 2 are shown in bold purple. Duplications of the B opsin gene have been observed twice in independent butterfly lineages: once in the pierid *P. rapae* giving rise to the violet receptor (PrV) (light purple) (Arikawa et al., 2005), and once in the lycaenid *L. rubidus*, giving rise to a blue–green (B2)-sensitive photopigment (BRh2; blue) (Sison-Mangus et al., 2006). (C) LW opsin gene tree. Opsin genes encoding the LW-absorbing visual pigments shown in Fig. 2 are indicated by bold orange, yellow and green. LW opsin gene duplications have occurred in three of five butterfly families independently. Besides those in *Papilio*, LW opsin duplicate genes have been recovered from the eye of the riodinid *Apodemia mormo* and from the genomic DNA of the nymphalid *Hermeuptychia hermes* (not shown) (Frentiu et al., 2007a). Scale bars indicate substitutions per site.

UV opsin in *Pieris* also follows that of the nymphalid in that it is found in either UV–UV or UV–B combinations in R1 and R2. In what appears to be a complete departure from the nymphalid ground plan for the eye, however, the B–B ommatidial subtype has been completely replaced in *P. rapae* ventral eye by a V–V ommatidial class that is the product of a B opsin gene duplication (Fig. 4). Since the violet-absorbing visual pigment is encoded by a duplicate B opsin (Fig. 3B), and the pattern of B opsin expression in other butterflies considered so far is restricted to the R1 and R2 cells, the V–V class of ommatidium can be interpreted as an example of subfunctionalization of the ancestral B opsin domain of expression, into a more limited expression pattern.

Subfunctionalization and neofunctionalization of B opsins in *Lycaena* has led to a sexually dimorphic eye

The pattern of duplicate B opsin expression in *Lycaena rubidus* illustrates both neofunctionalization and subfunctionalization and leads to the situation in which male and female butterflies literally see the world through different eyes. In the compound eye of this animal, four opsin genes are expressed – one UV (*UVRh*), duplicate B, B1 (*BRh1*) and B2 (*BRh2*), and one LW (*LWRh*) opsin (Fig. 3). It is the dorsal eye of *L. rubidus* that is sexually dimorphic. Unlike the papilionid, pierid or nymphalid eye, in which R3–8 express one or more LW opsins, in *L. rubidus* males, R3–8 exclusively express the B1 (dark blue ovals) opsin, while in females, R3–8 co-express the B1 and LW (dark blue–orange ovals) opsins (Fig. 4), an example of neofunctionalization of the B1 opsin domain of expression. In addition, the R1 and R2 cells of the dorsal eye of both males and females express UV–UV almost exclusively, with a minor number of ommatidia expressing the UV–B1 or B1–B1 combination (Sison-Mangus et al., 2006). The highly territorial male *L. rubidus* (Bernard and Remington, 1991) probably use their dorsal eye for dichromatic color vision and the detection of moving objects, such as airborne males.

The ventral eye, by contrast, has a pattern of LW opsin expression that is more consistent with the other butterfly families examined: R3–8 of the ventral retina express the LW opsin. It is here, though, that subfunctionalization of the second blue opsin duplicate gene, B2 (light blue ovals, Fig. 4), is evident. The ventral retina contains six classes of ommatidia that differ according to the opsins expressed in R1 and R2: UV–UV, UV–B1, UV–B2, B1–B2, B1–B1 or B2–B2 (Fig. 4).

Intriguingly, while butterflies in the genus *Papilio* use duplicate LW opsins to see green (Kelber, 1999), the lycaenid *Polyommatus icarus*, uses its duplicate B2 opsin, in conjunction with its LW opsin, to see in the green part of the spectrum extending up to 560 nm (Sison-Mangus et al., 2008). This suggests that natural selection has hit upon alternative strategies for color vision in the green range in lycaenid and papilionid butterflies.

Subfunctionalization and neofunctionalization of LW opsin expression in *Papilio*

The basic pattern of photoreceptor cell morphology and opsin mRNA expression exemplified by the nymphalid eye is also complicated in the swallowtail butterfly *Papilio* by alterations in the shapes of the photoreceptor cells and by the expression of the three duplicate LW opsins. For example, the ommatidium of the papilionid eye is organized into three tiers rather than two: a distal tier in which the cell bodies of the R1–4 cells are the widest, a middle tier in which the cell bodies of the R5–8 cells are the widest, and a third tier in which the cell body of the tiny R9 cell is located (Fig. 5).

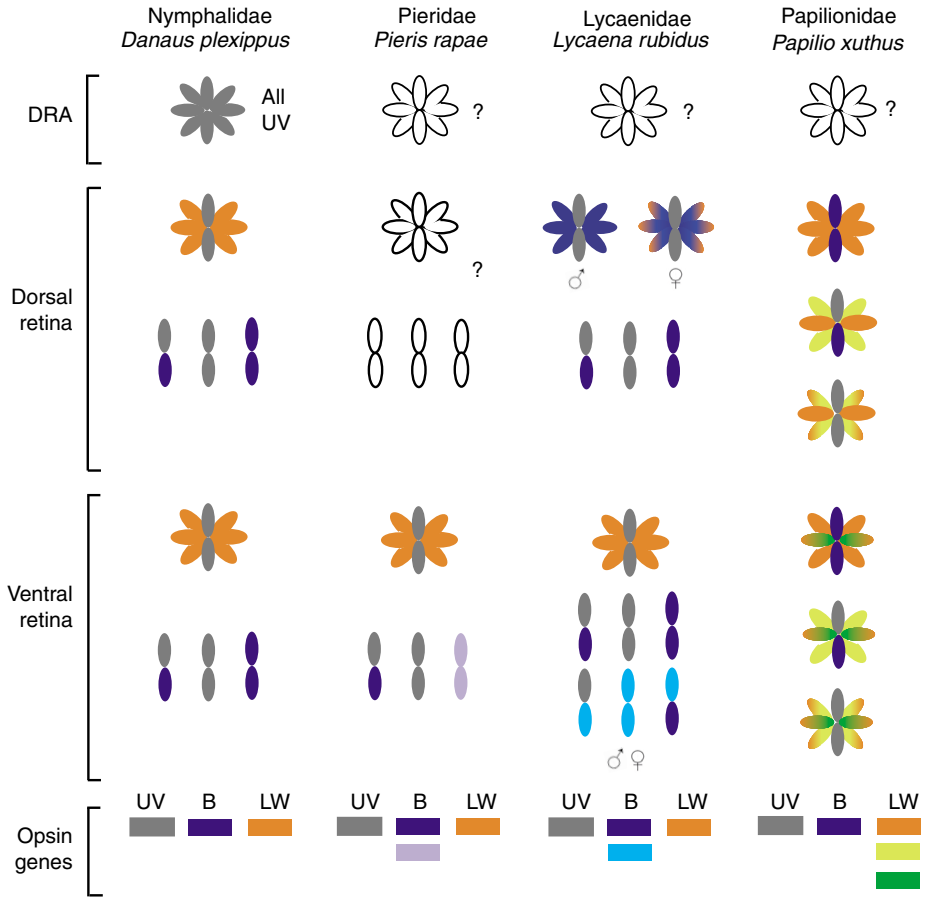

Fig. 4. Ommatidial subtypes found in the dorsal rim area (DRA), dorsal and ventral retina of butterflies according to opsin mRNA expression in individual R1–8 photoreceptor cells (ovals). Photoreceptor cells are color coded according to the identically colored visual pigments and opsins shown in Figs 2 and 3 that are expressed in them. Question mark indicates that opsin expression is unknown in some parts of the eyes reviewed here. Boxes indicate the UV, B and LW opsin genes and lineage-specific duplicates present in each species and are organized into columns according to membership in the UV, B or LW opsin clades shown in Fig. 2. Note: the patterns of opsin mRNA expression in the riodinid *Apodemia mormo* are unknown and so are not included in this scheme. Left to right: *D. plexippus* DRA is composed of approximately 100 ommatidia organized into two to three rows that exclusively express the UV opsin (gray) in the R1–8 cells. Both the dorsal and ventral eye contain three types of ommatidia in which the R1 and R2 cells express UV–UV, UV–B or B–B opsin mRNAs and the R5–8 cells express a LW opsin (orange) (Sauman et al., 2005). *P. rapae* ventral eye contains four opsins and three ommatidial subtypes in which R5–8 express one LW opsin mRNA (orange) and R1 and R2 express UV–B, UV–UV or V–V (violet) opsin mRNA (Arikawa et al., 2005). It is not yet known whether other ommatidial subtypes are present in the dorsal retina or in the DRA. *L. rubidus* dorsal eye contains three opsins and is sexually dimorphic with respect to their expression. In males, R5–8 exclusively expresses the B1 (purple) opsin, while in females the B1 opsin is co-expressed with the LW (orange) opsin. The R1 and R2 cells of the dorsal eye are almost entirely UV–UV, with the R1 and R2 cells of a few ommatidia expressing UV–B1 and even fewer expressing B1–B1. The ventral eye of both males and females contains one additional opsin, B2, encoded by a duplicate blue opsin gene, *BRh2*. The R3–8 cells all express the LW opsin and R1 and R2 all express UV–B1, UV–UV, B1–B1, UV–B2, B1–B2 or B2–B2 (Sison-Mangus et al., 2006). *P. xuthus* contains a dorsal eye with four opsins and three distinct ommatidial subtypes: B–B (dark purple) in R1 and R2 and PxRh2 (orange) in R3–8; UV–B in R1 and R2, PxRh2 in R3 and R4, and PxRh3 (yellow) in R5–8; UV–UV (gray) in R1 and R2, PxRh2 in R3 and R4, and co-expressed PxRh2 and PxRh3 (orange–yellow). The ventral retina contains five opsins and the same three ommatidial subtypes except PxRh1 is co-expressed with PxRh2 (green–orange) in all R3 and R4 cells in the ventral eye. Squares indicate opsin genes expressed in identically colored photoreceptor cells.

In *Papilio xuthus*, the butterfly eye about which we know the most, three LW opsin genes (*PxRh1*, *PxRh2* and *PxRh3*) are also expressed in the eye. In this butterfly, as in lycaenids, the main retina is divided into two parts: the dorsal eye and the ventral eye. Like nymphalids, the R1 and R2 cells of both the dorsal and ventral eye express UV–UV, UV–B or B–B opsin mRNAs. Unlike nymphalids, the R3 and R4 cells of the dorsal eye of *P. xuthus* express the *PxRh2* opsin mRNA (orange ovals, Fig. 4), while in the ventral eye, the R3 and R4 cells co-express *PxRh1* and *PxRh2* (green–orange ovals, Fig. 4) (Kitamoto et al., 1998).

Strikingly, the expression of opsins in R5–8 is coordinated with the expression of opsins in R1 and R2 (Arikawa, 2003). In the dorsal eye, one subtype contains B–B in R1 and R2 and *PxRh2* (orange ovals) in R5–8, another subtype contains UV–B in R1 and R2 and *PxRh3* (yellow ovals) in R5–8 and a third subtype contains UV–UV in R1 and R2 and *PxRh2* and *PxRh3* (orange–yellow) in R5–8. The ventral eye contains the same three ommatidial subtypes as in the dorsal eye, except as noted above, the *PxRh1* opsin is also co-expressed with the *PxRh2* opsin in R3 and R4. Altogether, there are at least six classes of ommatidia with respect to opsin expression in the main retina of *P. xuthus* (Fig. 4).

Interestingly, the most phylogenetically basal of the *P. xuthus* LW opsin gene duplicates expressed in the eye, *PxRh2* (Fig. 3), is

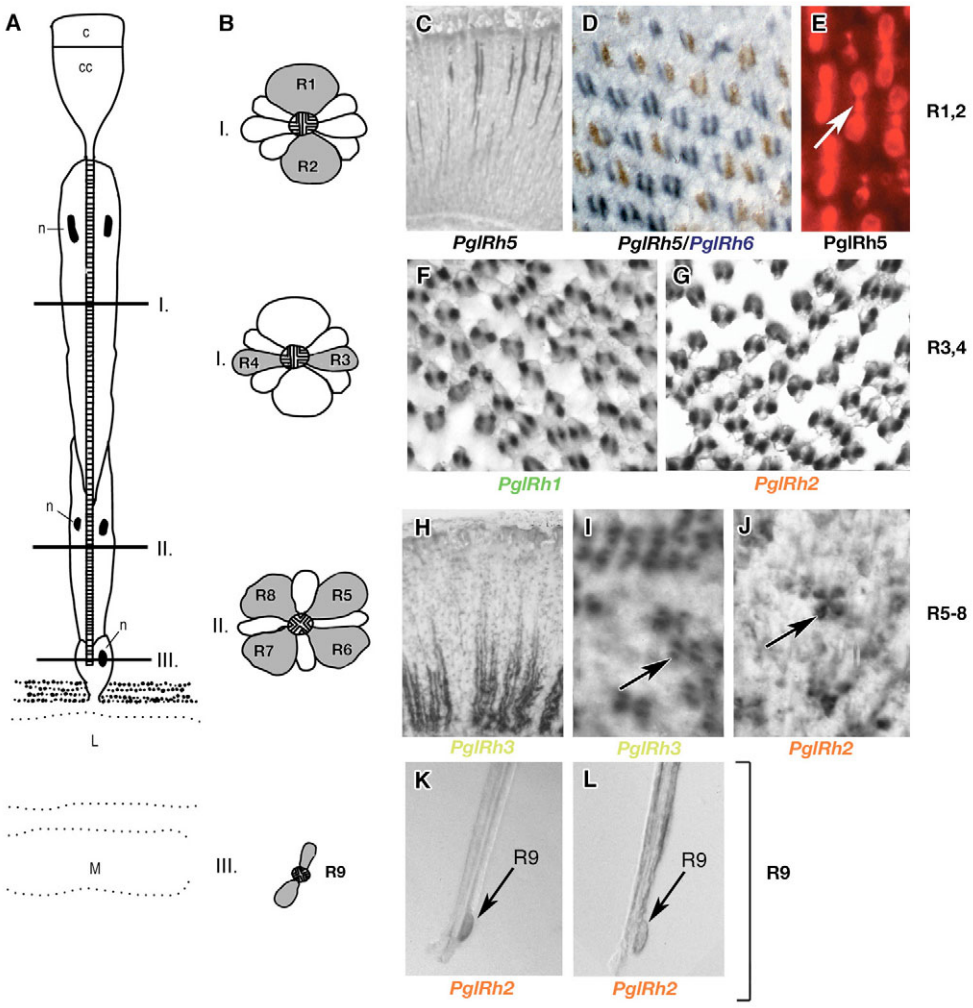

Fig. 5. Diagram of an ommatidium and opsin mRNA expression in the swallowtail butterfly *Papilio glaucus*, newly presented here. Methods used for the *in situ* hybridizations are as detailed previously (Briscoe et al., 2003). (A) Longitudinal view of an ommatidium. In *Papilio* spp., the photoreceptor cell bodies that contribute to the fused rhabdom are organized into three tiers: I, II and III. c, cornea; cc, crystalline cone; n, photoreceptor nucleus; L, lamina; M, medulla. (B) Tangential views of an ommatidium. The most distal tier (I) contains the cell bodies of the R1 and 2 and R3 and 4 cells. The proximal tier (II) contains the cell bodies of the R5–8 cells, and the basal tier (III) contains the R9 cell body. Gray indicates the identity of the photoreceptor cells in which specific opsin mRNA expression is shown in the panels to the right. (C) Digoxigenin-labeled antisense riboprobe of *UV* (*PglRh5*) indicating the expression of this opsin mRNA in the distal tier of photoreceptor cells. This opsin is homologous to the *UV* opsin gene of *P. xuthus*, *PxUV*, shown in Fig. 2. (D) A semi-tangential section showing double labeling of R1 and R2 cells with antisense biotin-labeled *UV* (*PglRh5*) (brown) and digoxigenin-labeled *B* opsin mRNA (*PglRh6*) (blue). The latter is homologous to the blue (B) opsin gene of *P. xuthus*, *PxB*, shown in Fig. 2. Three subtypes of ommatidia are evident: UV–UV, UV–B and B–B. (E) UV opsin expression (red) in R1 and R2 cells using a rabbit anti-PglRh5 peptide antibody generated by the author (see Lampel et al., 2005) and visualized with a Cy3-conjugated goat anti-rabbit secondary antibody. Tangential section showing opsin staining in both the rhabdom (white arrow) and cytoplasm. Two subtypes of ommatidia are shown here, UV–UV and UV–?, although all three subtypes implied by D are present elsewhere in the section. (F) Tangential section of the ventral retina showing staining of all R3 and R4 cells with a digoxigenin-labeled antisense *PglRh1* riboprobe. *PglRh1* is homologous to *P. xuthus PxRh1* shown in Fig. 2. (G) Tangential view of an adjacent section to F of the ventral retina showing staining of all R3 and R4 cells with digoxigenin-labeled antisense *PglRh2* riboprobe, and indicating co-expression of *PglRh1* and *PglRh2* in these cells in the ventral retina. In the dorsal eye, only *PglRh2* is present in the R3 and R4 cells (data not shown). (H) Longitudinal section of the eye indicating staining of the proximal (R5–8) tier of photoreceptor cells with a digoxigenin-labeled antisense riboprobe to *PglRh3*. (I) Tangential section showing strong staining of the R5–8 cells (arrow) of some ommatidia with a digoxigenin-labeled anti-sense riboprobe to *PglRh3*. (J) Tangential section showing strong staining of the R5–8 cells (arrow) of some ommatidia with a digoxigenin-labeled antisense riboprobe to *PglRh2*. (K) Dissociated ommatidium in which the R9 cell (arrow), but not the adjoining R5–8 cells, is clearly stained with a digoxigenin-labeled antisense riboprobe to *PglRh2*. This represents one (Type I) of three ommatidial subtypes defined by Arikawa (Arikawa, 2003). (L) Dissociated ommatidium in which both the R9 cell (arrow) and the R5–8 cells are clearly stained with a digoxigenin-labeled antisense riboprobe to *PglRh2*. This ommatidial subtype corresponds to either the Type II or Type III subtype (Arikawa, 2003).

also the one that has the widest expression in the R3–8 cells. And, like the LW opsin of *V. cardui* (Briscoe et al., 2003) and *P. glaucus* (Fig. 5K,L), it is also expressed in the R9 cell. This pattern is consistent with an ancestral function for *PxRh2* and a subfunctionalization of the more recent duplicates, *PxRh1* and *PxRh3* (Briscoe, 2000), into a more limited domain of expression. It is also worth noting that *PxRh3* exemplifies neofunctionalization, as it encodes P575, whose absorbance spectrum maximum is quite red shifted compared with *PxRh2*, which encodes P530 [see discussion in Briscoe (Briscoe, 2000)].

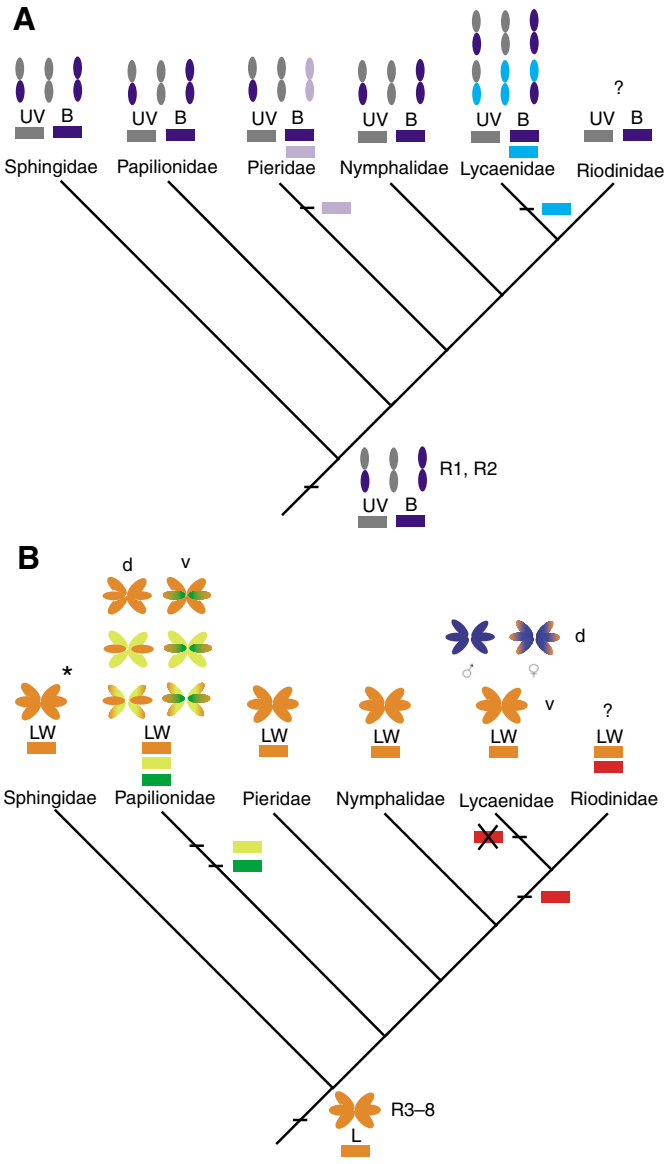

Fig. 6. Phylogeny of the butterfly families, character mapping of opsin mRNA expression patterns in individual photoreceptor cell subtypes, and proposed ancestral butterfly eye. Question mark indicates unknown opsin expression in the photoreceptor cells of the riodinid eye. (A) Character mapping of opsin mRNA expression in the R1 and R2 cells and inferred relative timing of opsin gene duplication events. Ovals indicate UV (gray) or B (purple, violet, blue) opsin expression. Boxes and ovals indicate the inferred ancestral states along specific branches of the butterfly family tree for both opsin genes and expression patterns. The R1 and R2 opsin expression patterns are not known for the Riodinidae. (B) Character mapping of opsin expression in the R3–8 cells. Ovals indicate photoreceptor cells expressing LW (orange, yellow or green) or B (purple) opsin mRNAs. Asterisk indicates ventral eye only. Red box indicates a LW opsin duplicate, the precursor to *Apodemia mormo* LWRh1, that is inferred to have arisen prior to the radiation of the lycaenid and riodinid families, and was subsequently lost in lycaenids. The R3–8 opsin expression patterns are not known for the Riodinidae.

Filtering pigments modify the sensitivity of photoreceptor cells to light

In addition to the visual pigments, all four butterfly eyes profiled here contain one or more combinations of yellow, orange or red filtering pigments (Arikawa et al., 1999b; Arikawa, 2003; Stavenga and Arikawa, 2006). Even more remarkable is that the eye of *Pieris rapae crucivora* is sexually dimorphic with respect to the expression of a blue-absorbing filtering pigment that is present only in the eye of males and is co-expressed with the violet opsin (Arikawa et al., 2005). Moreover, some butterfly eyes not reviewed here lack filtering pigments entirely (Briscoe and Bernard, 2005). Lateral filtering pigments coat the rhabdom, and filter short wavelength light, thus shifting the sensitivity of the visual pigments to the longer wavelengths. Examples of such an effect for red filtering pigments have been shown electrophysiologically in *Heliconius erato* (Bernhard et al., 1970; Swihart and Gordon, 1971; Struwe, 1972), *Papilio xuthus* (Arikawa et al., 1987; Arikawa et al., 1999a) and *Pieris rapae crucivora* (Qiu et al., 2002; Wakakuwa et al., 2004), and for the blue-absorbing filtering pigment of *P. rapae* (Arikawa et al., 2005).

The biological significance of filtering pigments is that while they do not expand the total visual range of the animal (this is dependent on the visual pigments themselves), they may expand the animal's color vision range. The red filtering pigments in the eyes of butterflies, however, do not appear to have a unified function. For instance, behavioral tests have shown that the nymphalid *Heliconius erato* uses a heterogeneously expressed red filtering pigment together with a single LW opsin to produce expanded color vision in the long wavelength range when foraging (Zaccardi et al., 2006), but the lycaenid *Polyommatus icarus*, which also has a heterogeneously expressed red filtering pigment in its eye, does not appear to have expanded color vision, at least when tested in the context of feeding (Sison-Mangus et al., 2008).

Providing a clearer role for filtering pigments in color vision will require further behavioral testing in a larger collection of butterfly species and, in some cases, such as the red receptor of *Papilio*, which co-expresses a red-filtering pigment with a red-absorbing visual pigment (Arikawa, 2003), it will be difficult to disentangle the impact of the filtering pigment on color vision from that of their coordinately expressed opsin.

Reconstructing the ancestral butterfly eye

The phylogeny of the butterflies provides a framework for reconstructing the pattern of opsin expression in the ancestral eye. Within the true butterflies (Papilionoidea) the current understanding of familial relationships is Papilionidae+{Pieridae+[Nymphalidae+(Lycaenidae+Riodinidae)]} (Wahlberg et al., 2005), where papilionid and pierid butterflies represent the most basal lineages, and nymphalid, lycaenid and riodinid the most derived (Fig. 6). The inferred instances of lineage-specific opsin gene duplications (Fig. 3) together with the extant pattern of opsin mRNA expression (summarized in Fig. 4) can be mapped onto the butterfly family species tree to infer the most parsimonious ancestral state, using the sphingid moth *Manduca sexta* as an outgroup. Remarkably, the ancestral state is not represented by the most basal butterfly lineages but, instead, is manifested in the nymphalids.

Fig. 6A shows the most parsimonious reconstruction of the ancestral pattern of opsin mRNA expression in the R1 and R2 cells. Using this reconstruction, the ancestor of all butterfly eyes had merely one UV (gray box and ovals) and one B opsin (dark blue box and ovals) gene expressed in a non-overlapping fashion in the R1 and R2 cells – a pattern shared with the opsin expression in the main retina of sphingid moths (White et al., 2003) and bees (Spaethe and Briscoe, 2005; Wakakuwa et al., 2005) and consistent with the existence of only one UV and one B opsin gene in the genome of the silkmoth, *Bombyx mori* (Mita et al., 2004; Xia et al., 2004).

Subsequently, sometime after the divergence of the pierid from the papilionid and nymphalid+(lycaenid+riodinid) lineages, a B

opsin gene duplication arose, presumably prior to the radiation of the Pierinae+Coliadinae subfamilies, since a homologue of the *P. rapae* (Pierinae) V opsin has been cloned from *Colias philodice* (Sison-Mangus et al., 2006), a species in one of the other major pierid subfamilies, Coliadinae. [The two more basal subfamilies, Pseudopontiinae and Dismorphiinae (Braby et al., 2006; Chew and Watt, 2006) have not yet been assayed for opsins.]

The pattern of ancestral LW opsin expression can be reconstructed similarly, and doing so suggests that the common ancestor of all butterfly eyes expressed a single LW opsin in the R3–8 cells (and also probably in the R9 cell) (Fig. 6B). Under this scenario, sometime after the split between the lineage leading to *Papilio* and the other butterfly families, two rounds of LW opsin gene duplication occurred, followed by subfunctionalization of the newer paralogs in *Papilio*. Intriguingly, the topology of the LW opsin gene tree also implies that the LW opsin duplicated prior to the radiation of the riodinid and lycaenid subfamilies, and that the homolog of the riodinid LWRh1 opsin was lost in the lineage leading to lycaenids. Subsequently, in the case of the highly divergent, sexually dimorphic pattern of opsin expression in the R3–8 cells of *L. rubidus*, the co-expression of a B opsin, B1 in R3–8, along with the LW opsin, can be viewed as an intermediate step along the evolution of a new function for B1 opsin in the dorsal eye of males (Sison-Mangus et al., 2006).

An eye to the future

One could imagine extending ancestral state reconstruction to other parts of the butterfly visual system to further understand the evolution of photoreceptor patterning, color vision, and even an integration of the evolution of the visual system with wing color. For instance, at present, the molecular identity, phylogenetic distribution and behavioral impact of filtering pigments in the eyes of butterflies are not well understood, but it seems likely that the ancestral butterfly eye contained heterogeneously expressed lateral filtering pigments, with secondary losses. Like the opsins, lateral filtering pigments may have evolved lineage-specific modifications and expression patterns that could have an impact on the color vision system. And, as has been pointed out, some of the pigments found in the brightly colored scales of butterfly wings are evolutionarily derived from eye pigments (Nijhout, 1991; Reed and Nagy, 2005). So it is possible that modifications of wing pigments co-evolved with the modifications of eye pigments.

Similarly, it is not known how the dorsal rim area ommatidium of the ancestral butterfly eye was patterned with respect to opsin expression. Unlike most moths, which are primarily nocturnal and may use polarized moonlight or starlight for navigation, butterflies are diurnally active and use the polarized skylight as a navigational cue (Froy et al., 2003; Reppert et al., 2004). If different opsins are expressed in the DRA ommatidia of different butterflies, this would imply that the polarized skylight cues used by butterflies are not uniform. In fact, it would not be surprising if this were the case, given the diversity of spectral classes of photoreceptors found in the DRA ommatidia of different insects (Labhart and Meyer, 1999) and the apparent ease with which domains of opsin expression in butterflies have been altered. A hint that the DRA ommatidia themselves may have evolved in Lepidoptera is evident in the observation that while the R1–8 cells in monarch DRA ommatidia express only the UV opsin (Sauman et al., 2005), in the hawkmoth, *Manduca sexta*, only some of the photoreceptor cells in the DRA ommatidia express the UV opsin (P357), while other photoreceptor cells express none of the other (B and L) opsins cloned (White et al., 2003).

Finally, alterations in opsin expression patterns beg the question of whether or not the neural circuitry necessary to process this information has evolved. At present, correlation between opsin expression and photoreceptor axon terminals has only been attempted in the swallowtail (Takemura et al., 2005; Takemura and Arikawa, 2006). The collateral morphology of the axons of the R5–8 cells of *P. xuthus* varies according to which LW opsin they express. But what about in other butterflies that do not have duplicated LW opsins?

In conclusion, it is clear that the butterfly eye is one of the most beautifully honed instruments of evolution. Bringing to bear molecular tools that are now becoming available for non-model organisms (e.g. genetic transformation, custom microarrays and RNA interference) will allow a deeper understanding of the biological events involved in butterfly eye diversity that may be applicable to a broader range of biological processes.

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References

Arikawa, K. (2003). Spectral organization of the eye of a butterfly, *Papilio*. *J. Comp. Physiol. A* **189**, 791-800.
Arikawa, K. and Stavenga, D. G. (1997). Random array of colour filters in the eyes of butterflies. *J. Exp. Biol.* **200**, 2501-2506.
Arikawa, K., Inokuma, K. and Eguchi, E. (1987). Pentachromatic visual system in a butterfly. *Naturwissenschaften* **74**, 297-298.
Arikawa, K., Scholten, D. G. W., Kinoshita, M. and Stavenga, D. G. (1999a). Tuning of photoreceptor spectral sensitivities by red and yellow pigments in the butterfly *Papilio xuthus*. *Zool. Sci.* **16**, 17-24.
Arikawa, K., Mizuno, S., Scholten, D. G. W., Kinoshita, M., Seki, T., Kitamoto, J. and Stavenga, D. G. (1999b). 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., Wakakuwa, M., Qiu, X., Kurasawa, M. and Stavenga, D. G. (2005). Sexual dimorphism of short-wavelength photoreceptors in the small white butterfly, *Pieris rapae crucivora*. *J. Neurosci.* **25**, 5935-5942.
Bernard, G. D. and Remington, C. L. (1991). Color vision in *Lycaena* butterflies: spectral tuning of receptor arrays in relation to behavioral ecology. *Proc. Natl. Acad. Sci. USA* **88**, 2783-2787.
Bernhard, C. G., Boethius, J., Gemne, G. and Struwe, G. (1970). Eye ultrastructure, colour reception and behaviour. *Nature* **226**, 865-866.
Braby, M. F., Vila, R. and Pierce, N. E. (2006). Molecular phylogeny and systematics of the Pieridae (Lepidoptera: Papilionoidea): higher classification and biogeography. *Zool. J. Linn. Soc.* **147**, 239-275.
Briscoe, A. D. (1998). Molecular diversity of visual pigments in the butterfly *Papilio glaucus*. *Naturwissenschaften* **85**, 33-35.
Briscoe, A. D. (2000). Six opsins from the butterfly *Papilio glaucus*: molecular phylogenetic evidence for paralogous origins of red-sensitive visual pigments in insects. *J. Mol. Evol.* **51**, 110-121.
Briscoe, A. D. (2001). Functional diversification of lepidopteran opsins following gene duplication. *Mol. Biol. Evol.* **18**, 2270-2279.
Briscoe, A. D. and Bernard, G. D. (2005). Eyeshine and spectral tuning of long wavelength-sensitive rhodopsins: no evidence for red-sensitive photoreceptors among five Nymphalini butterfly species. *J. Exp. Biol.* **208**, 687-696.
Briscoe, A. D. and Chittka, L. (2001). The evolution of color vision in insects. *Annu. Rev. Entomol.* **46**, 471-510.
Briscoe, A. D., Bernard, G. D., Szeto, A. S., Nagy, L. M. and White, R. H. (2003). Not all butterfly eyes are created equal: rhodopsin absorption spectra, molecular identification and localization of ultraviolet-, blue-, and green-sensitive rhodopsin-encoding mRNAs in the retina of *Vanessa cardui*. *J. Comp. Neurol.* **458**, 334-349.
Chew, F. S. and Watt, W. B. (2006). The green-veined white (*Pieris napi* L.), its Pierine relatives, and the systematics dilemmas of divergent character sets (Lepidoptera, Pieridae). *Biol. J. Linn. Soc. Lond.* **88**, 413-435.
Force, A., Lynch, M. K., Pickett, F., Amores, A., Yan, Y. L. and Postlethwait, J. (1999). Preservation of duplicate genes by complementary, degenerative mutations. *Genetics* **151**, 1531-1545.
Frentiu, F. D., Bernard, G. D., Sison-Mangus, M. P., Brower, A. V. Z. and Briscoe, A. D. (2007a). Gene duplication is an evolutionary mechanism for expanding spectral diversity in the long wavelength photopigments of butterflies. *Mol. Biol. Evol.* **24**, 2016-2028.
Frentiu, F. D., Bernard, G. D., Cuevas, C. I., Sison-Mangus, M. P., Prudic, K. L. and Briscoe, A. D. (2007b). Adaptive evolution of color vision as seen through the eyes of butterflies. *Proc. Natl. Acad. Sci. USA* **104** Suppl. 1, 8634-8640.
Froy, O., Gotter, A. L., Casselman, A. L. and Reppert, S. M. (2003). Illuminating the circadian clock in monarch butterfly migration. *Science* **300**, 1303-1305.
Honig, B., Greenberg, A. D., Dinur, U. and Ebrey, T. G. (1976). Visual-pigment spectra: implications of the protonation of the retinal Schiff base. *Biochemistry* **15**, 4593-4599.

Kelber, A. (1999). Ovipositing butterflies use a red receptor to see green. *J. Exp. Biol.* **202**, 2619-2630.
Kelber, A. and Pfaff, M. (1999). True colour vision in the orchard butterfly, *Papilio aegeus*. *Naturwissenschaften* **86**, 221-224.
Kinoshita, M., Shimada, N. and 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. and Arikawa, K. (1998). Two visual pigments in a singe 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. and 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.
Labhart, T. and Meyer, E. P. (1999). Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microsc. Res. Tech.* **47**, 368-379.
Lampel, J., Briscoe, A. D. and Wasserthal, L. T. (2005). Expression of UV-, blue-, long-wavelength-sensitive opsins and melatonin in extraretinal photoreceptors of the optic lobes of hawkmoths. *Cell Tissue Res.* **321**, 443-458.
Mita, K., Kasahara, M., Sasaki, S., Nagayasu, Y., Yamada, T., Kanamori, H., Namiki, N., Kitagawa, M., Yamashita, H., Yasukochi, Y. et al. (2004). The genome sequence of silkworm, *Bombyx mori*. *DNA Res.* **11**, 27-35.
Nijhout, H. (1991). *The Development and Evolution of Butterfly Wing Patterns*. Washington: Smithsonian Institution Press.
Palacios, A. G., Goldsmith, T. H. and Bernard, G. D. (1996). Sensitivity of cones from a cyprinid fish (*Danio aequipinnatus*) to ultraviolet and visible light. *Vis. Neurosci.* **13**, 411-421.
Peitsch, D., Feitz, A., Hertel, H., de Souza, J., Ventura, D. and Menzel, R. (1992). The spectral input systems of hymenopteran insects and their receptor-based colour vision. *J. Comp. Physiol. A* **170**, 23-40.
Qiu, X. and Arikawa, K. (2003a). 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. and Arikawa, K. (2003b). The photoreceptor localization confirms the spectral heterogeneity of ommatidia in the male small white butterfly, *Pieris rapae crucivora*. *J. Comp. Physiol. A* **189**, 81-88.
Qiu, X., Vanhoutte, K. A., Stavenga, D. G. and Arikawa, K. (2002). Ommatidial heterogeneity in the compound eye of the male small white butterfly, *Pieris rapae crucivora*. *Cell Tissue Res.* **307**, 371-379.
Reed, R. D. and Nagy, L. M. (2005). Evolutionary redeployment of a biosynthetic module: expression of eye pigment genes *vermilion, cinnabar*, and *white* in butterfly wing development. *Evol. Dev.* **7**, 301-311.
Reppert, S. M., Zhu, H. and White, R. H. (2004). Polarized light helps monarch butterflies navigate. *Curr. Biol.* **14**, 155-158.
Sauman, I., Briscoe, A. D., Zhu, H., Shi, D., Froy, O., Stalleicken, J., Yuan, Q., Casselman, A. and Reppert, S. M. (2005). Connecting the navigational clock to sun compass input in monarch butterfly brain. *Neuron* **46**, 457-467.
Shimizu, I., Yamakawa, Y., Shimazaki, Y. and Iwasa, T. (2001). Molecular cloning of *Bombyx* cerebral opsin (*Boceropsin*) and cellular localization of its expression in the silkworm brain. *Biochem. Biophys. Res. Commun.* **287**, 27-34.
Sison-Mangus, M. P., Bernard, G. D., Lampel, J. and Briscoe, A. D. (2006). Beauty in the eye of the beholder: the two blue opsins of lycaenid butterflies and the opsin gene-driven evolution of sexually dimorphic eyes. *J. Exp. Biol.* **209**, 3079-3090.
Sison-Mangus, M. P., Briscoe, A. D., Zaccardi, G., Knüttel, H. and Kelber, A. (2008). The lycaenid butterfly *Polyommatus icarus* uses a duplicated blue opsin to see green. *J. Exp. Biol.* **211**, 361-369.
Smith, C. W. and Goldsmith, T. H. (1990). Phyletic aspects of the distribution of 3-hydroxyretinal in the Class Insecta. *J. Mol. Evol.* **30**, 72-84.
Spaethe, J. and Briscoe, A. D. (2005). Molecular characterization and expression of the UV opsin in bumblebees: three ommatidial subtypes in the retina and a new photoreceptor organ in the lamina. *J. Exp. Biol.* **208**, 2347-2361.
Stalleicken, J., Labhart, T. and Mouritsen, H. (2006). Physiological characterization of the compound eye in monarch butterflies with focus on the dorsal rim area. *J. Comp. Physiol. A* **192**, 321-331.
Stavenga, D. G. (2002). Reflections on colourful ommatidia of butterfly eyes. *J. Exp. Biol.* **205**, 1077-1085.
Stavenga, D. G. and Arikawa, K. (2006). Evolution of color and vision of butterflies. *Arthropod Struct. Dev.* **35**, 307-318.
Stavenga, D. G., Smits, R. P. and Hoenders, B. J. (1993). Simple exponential functions describing the absorbance bands of visual pigment spectra. *Vision Res.* **33**, 1011-1017.
Struwe, G. (1972). Spectral sensitivity of the compound eye in butterflies (*Heliconius*). *J. Comp. Physiol.* **79**, 191-196.
Swihart, S. and Gordon, W. (1971). Red photoreceptor in butterflies. *Nature* **231**, 126-127.
Takemura, S. Y. and Arikawa, K. (2006). Ommatidial type-specific interphotoreceptor connections in the lamina of the swallowtail butterfly, *Papilio xuthus*. *J. Comp. Neurol.* **494**, 663-672.
Takemura, S. Y., Kinoshita, M. and Arikawa, K. (2005). Photoreceptor projection reveals heterogeneity of lamina cartridges in the visual system of the Japanese yellow swallowtail butterfly, *Papilio xuthus*. *J. Comp. Neurol.* **483**, 341-350.
Wahlberg, N., Braby, M. F., Brower, A. V. Z., de Jong, R., Lee, M. M., Nylin, S., Pierce, N. E., Sperling, F. A. H., Vila, R., Warren, A. D. et al. (2005). Synergistic effects of combining morphological and molecular data in resolving the phylogeny of butterflies and skippers. *Proc. R. Soc. Lond. B Biol. Sci.* **272**, 1577-1586.
Wakakuwa, M., Stavenga, D. G., Kurasawa, M. and Arikawa, K. (2004). A unique visual pigment expressed in green, red and deep-red receptors in the eye of the small white butterfly, *Pieris rapae crucivora*. *J. Exp. Biol.* **207**, 2803-2810.
Wakakuwa, M., Kurasawa, M., Giurfa, M. and Arikawa, K. (2005). Spectral heterogeneity of honeybee ommatidia. *Naturwissenschaften* **92**, 464-467.
White, R. H., Xu, H. H., Munch, T. A., Bennett, R. R. and Grable, E. A. (2003). The retina of *Manduca sexta:* rhodopsin expression, the mosaic of green-, blue- and UV-sensitive photoreceptors, and regional specialization. *J. Exp. Biol.* **206**, 3337-3348.
Xia, Q., Zhou, Z., Lu, C., Cheng, D., Dai, F., Li, B., Zhao, P., Zha, X., Cheng, T., Chai, C. et al. (2004). A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). *Science* **306**, 1937-1940.
Zaccardi, G., Kelber, A., Sison-Mangus, M. P. and Briscoe, A. D. (2006). Color discrimination in the red range with only one long-wavelength sensitive opsin. *J. Exp. Biol.* **209**, 1944-1955.