

Chapter 5

Insect Photopigments: Photoreceptor Spectral Sensitivities and Visual Adaptations

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Abstract The spectral sensitivity of photoreceptors is primarily determined by the expressed rhodopsins. After a brief introduction to the photochemistry of insect rhodopsins, the relatively simple case of bee visual pigments and photoreceptors is described, followed by the more complicated cases of butterflies and flies. Although the main focus is on the properties of visual pigments, considerable attention is also given to other photostable filter pigments that importantly modify the spectral properties of the photoreceptors. The sexual dimorphism of the filter pigments results in the sexual dimorphism of photoreceptor spectral sensitivities.

Keywords Color vision • Compound eye • Ommatidium • Photoreceptor • Rhabdom • Spectral sensitivity • Sensitizing pigment • Filter pigment • Fluorescent pigment • Sexual dimorphism

Abbreviations

S	Short wavelength-absorbing
M	Middle wavelength-absorbing
L	Long wavelength-absorbing
R1–R9	Photoreceptor 1–9

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5.1 Introduction

Visual pigment diversity is under evolutionary pressure because the visual pigments are the essential elements that determine the spectral sensitivity of the photoreceptors. A photoreceptor set with different spectral sensitivities serves as the physiological basis of color vision. The spectral environment and visual tasks thus have to be considered in close connection with the visual pigments' spectral properties.

The study of insect visual pigments essentially started about a hundred years ago when Karl von Frisch published his pioneering work on the color vision of the European honeybee, *Apis mellifera* (Frisch 1914). Since then insect color vision has become a central topic in biology, making von Frisch in 1973, together with Konrad Lorenz and Nikolaas Tinbergen, a Nobel laureate for fundamental contributions to the study of animal behavior.

Because of the influence of von Frisch, honeybees have been intensely studied ever since. Fifty years after the initial color vision studies, Autrum and von Zwehl, applying intracellular electrophysiology to insect photoreceptors for the first time, reported four distinct spectral sensitivities of single photoreceptors in the worker honeybee (Autrum and von Zwehl 1964). The number of spectrally different photoreceptors were later adjusted to three, with sensitivities peaking at around 350 nm (ultraviolet, UV), 440 nm (blue, B) and 540 nm (green, G) (Menzel and Blakers 1976). These three spectral receptor classes provide the physiological basis for the trichromatic system of honeybees (Daumer 1956; von Helversen 1972). It is strikingly similar to the human trichromatic system with a notion that the visible light range of insects is shifted about 100 nm toward the shorter wavelengths.

Due to the emphasis on honeybee research, studies on other insect species were initially largely ignored (Ilse 1941). Extensive comparative research started in the late 1960s (Bennett et al. 1967; Menzel 1979), and flourished in the 1980s. The outcome of those studies later established that insect photoreceptors can have quite variable spectral sensitivities, depending on the species (Eguchi et al. 1982, 1984; Matic 1983; Arikawa et al. 1987). Stimulated by the early electrophysiological studies, *in vitro* and *in situ* spectroscopy was applied in the 1970s to understand the physiological nature of visual pigments (Hamdorf 1979).

Subsequently, in the 1980s, molecular biology became widely applicable. The primary structures of some mammalian visual pigment opsins were first identified (Hargrave et al. 1983; Nathans and Hogness 1983; Nathans et al. 1986), rapidly followed by the analysis of the visual pigments of the fruitfly *Drosophila melanogaster* (Zuker et al. 1985, 1987). Next, three opsins of honeybees were cloned in the 1990s (Chang et al. 1996; Bellingham et al. 1997; Townson et al. 1998), which was followed by the sequence data of opsins from an increasing number of insect orders, totaling to date more than 2,000 visual pigment molecules. The data clearly indicate that insect opsins collectively form three clades: short wavelength (S) or UV-absorbing, middle wavelength (M) or blue-absorbing, and long wavelength (L) or green-absorbing visual pigments (Fig. 5.1).

The accumulated information about visual pigments provides a broadly coherent view about their molecular and photochemical properties. Furthermore, it has

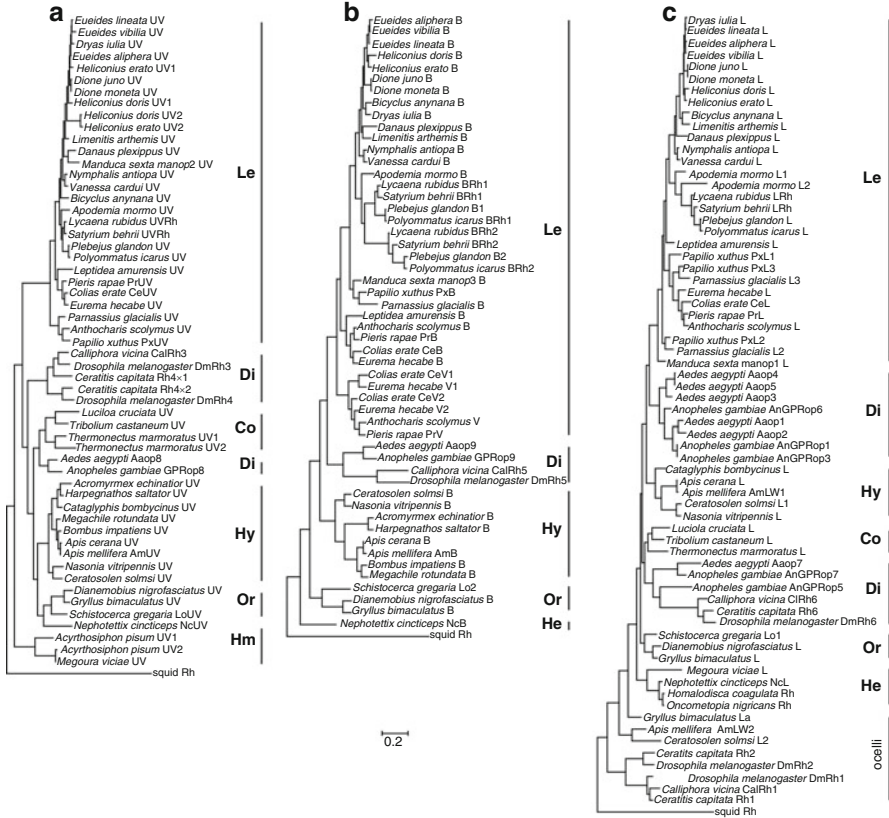


Fig. 5.1 Molecular phylogeny of the three clades of insect opsins. (a) Short wavelength (S) or UV-absorbing type. (b) Middle wavelength (M) or blue-absorbing. (c) Long wavelength (L) or green-absorbing

promoted an understanding of the physiological mechanisms underlying photoreceptor spectral sensitivities. Insect compound eyes thus have become to be a rich source for studies of visual specializations. For instance, compound eyes often show a distinct regionalization as well as sexual dimorphism, affecting the expression of the visual pigments and consequently the photoreceptor spectral sensitivities. Non-visual, so-called screening pigments may also play a distinct role in modifying the spectral sensitivities.

The spectral sensitivity of photoreceptors is primarily determined by the expressed visual pigments. After a brief introduction to the photochemistry of insect visual pigments, the relatively simple case of bees (order Hymenoptera) will be presented, followed by more complicated cases, particularly those of butterflies (order Lepidoptera) and flies (Diptera). Although the main focus will be on the properties of visual pigments, considerable attention will be given to other pigments that importantly modify the spectral properties of the photoreceptors.

5.2 Photochemistry of Insect Visual Pigments

A visual pigment molecule consists of two components, an opsin protein and a vitamin A aldehyde attached to opsin as the chromophore. Commonly in vertebrates the chromophore is 11-*cis* retinal, but many insects employ 11-*cis* 3-hydroxy-retinal (Vogt and Kirschfeld 1983). Visual pigments with different chromophore molecules are sometimes given different names, but for simplicity we here use the term rhodopsin for all visual pigments, independent of the type of chromophore.

When a rhodopsin molecule absorbs a photon, the chromophore is isomerized into the all-*trans* form. The quantum efficiency of the conversion has not been measured for any insect rhodopsin, but it may be similar to that determined for bovine rhodopsin, which is about two-thirds (Dartnall 1972). The isomerization of the chromophore subsequently causes the transformation of the whole pigment molecule, via a few thermally unstable intermediates, to metarhodopsin. In the case of vertebrate visual pigments, metarhodopsin further decays, but in invertebrates it is thermally stable (Gärtner 2000).

The metarhodopsins of insects have their own distinct absorption spectra, so that photon absorption by metarhodopsin can re-isomerize the all-*trans* chromophore back into the 11-*cis* form. Prolonged exposure of insect visual pigments to monochromatic light hence creates a photosteady state that depends on the ratio of the absorption coefficients of the two states, rhodopsin and metarhodopsin.

The photochemical steps are often accompanied by both shifts of the absorption peak wavelength and distinct changes in peak absorption. By measuring absorbance difference spectra, the photochemical processes can be studied even in turbid or complex tissues containing other pigments, and even in the living eye (Hamdorf 1979).

5.3 The UV Pigment of the Owlfly *Libelloides macaronius*

The intermediate states in the photochemical cycle of both vertebrate and invertebrate visual pigments are thermostable below a certain critical temperature, and they can thus be studied sequentially by photoconversion of rhodopsin at appropriately chosen temperatures. These low temperature studies are preferentially performed on visual pigment extracts. As an example, a few photochemical steps are shown for the UV-rhodopsin of the owlfly *Libelloides* (formerly *Ascalaphus*) *macaronius* (Neuroptera), the first lucid example of an UV-absorbing visual pigment (Fig. 5.2; Belušič et al. 2013). Upon illumination the pigment can attain various states, as witnessed by their different absorbance spectra. The owlfly rhodopsin absorbs maximally at 345 nm (Hamdorf 1979). Absorption of a photon by rhodopsin (R) at $-50\text{ }^{\circ}\text{C}$ yields lumirhodopsin (L). In this state the visual pigment absorbs much stronger than the native rhodopsin, indicative of the 11-*cis* all-*trans* isomerization of the chromophore, and the peak wavelength is shifted to about 375 nm. The lumirhodopsin can be photoconverted back to rhodopsin at $-50\text{ }^{\circ}\text{C}$, but it is unstable

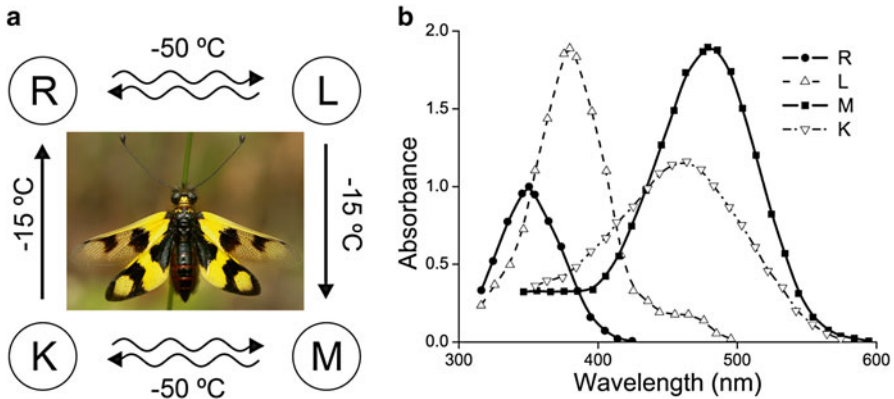


Fig. 5.2 Photochemical cycle and spectral characteristics of the UV-absorbing rhodopsin and its photoproducts in the owlfly *Libelloidus macaronius*. (a) At low temperatures ($-50\text{ }^{\circ}\text{C}$) photon absorption results in conversion of rhodopsin, R, to lumirhodopsin, L, which can be photoconverted back into rhodopsin. Upon warming ($-15\text{ }^{\circ}\text{C}$) lumirhodopsin transforms to metarhodopsin, M. At low temperatures this is photointerconvertible with an intermediate, K, which above $-15\text{ }^{\circ}\text{C}$ decays to the rhodopsin state. The chromophores in R and K take the 11-*cis* configuration, and the chromophores in L and M are all-*trans*. (b) Absorbance spectra normalized to the rhodopsin peak absorbance of the various visual pigment states (modified from Hamdorf 1979)

at $-15\text{ }^{\circ}\text{C}$. The end photoproduct, metarhodopsin (M), then results (Fig. 5.2). The strong bathochromic shift of the peak wavelength indicates distinct intramolecular conformation changes. As in lumirhodopsin, the peak absorbance coefficient of metarhodopsin is about 1.8 times that of rhodopsin.

Irradiation of metarhodopsin at $-50\text{ }^{\circ}\text{C}$ yields an intermediate K (Fig. 5.2), which is photointerconvertible with metarhodopsin. The strongly reduced absorbance coefficient signifies the all-*trans* to 11-*cis* isomerization, but the peak wavelength shift to 460 nm suggests relatively minor changes in the conformation of the whole protein. At $-15\text{ }^{\circ}\text{C}$ intermediate K decays thermally to rhodopsin.

Low temperature spectroscopy of visual pigments and their intermediates has been performed in several vertebrates and a few invertebrates (Yoshizawa 1972; Hamdorf 1979; Vought et al. 2000). The decay scheme appears to follow a rather uniform temporal pattern, at least in the pathway of vertebrates, where rhodopsin transforms via bathorhodopsin, lumirhodopsin, and metarhodopsin to retinal and opsin. The photoconversion of the intermediates, demonstrated to occur at low temperatures, occurs much more rapidly at physiological temperatures. However, due to the brief lifetimes of the intermediates, it then requires extreme irradiation intensities to cause noticeable photoconversions, and the chance of photoconverting intermediates is negligible at light fluxes existing under normal, physiological conditions. For all general spectral considerations, it is therefore adequate to consider insect visual pigments to exist either in the rhodopsin or the metarhodopsin configurations.

5.4 Bee Visual Pigments

The compound eyes of honeybees consist of about 6,000 ommatidia, each containing nine photoreceptor cells, R1–R9. Eight of them (R1–R8) are elongated cells that extend over the full retinal layer of the ommatidium, while R9 is a small photoreceptor at the base of the ommatidium. Each photoreceptor extends microvilli that form a visual pigment-containing rhabdomere. The rhabdomeres of a honeybee ommatidium are closely apposed, forming a fused rhabdom that acts as a single optical waveguide (Fig. 5.3b).

As suggested by the phylogeny of visual pigment opsins (Fig. 5.1), the eye of the honeybee has three photoreceptor classes, UV, B and G (Fig. 5.3c), each expressing a specific opsin: AmUV (*Apis mellifera* UV), AmB, and AmL (Fig. 5.3d–f). The spectral sensitivities of honeybee photoreceptors thus can be explained by the different absorption spectra of the three visual pigments. The absorption spectra of the honeybee visual pigments were first estimated from electrophysiological recordings, but they were more accurately assessed by ectopically expressing the opsins in *Drosophila* (Townson et al. 1998).

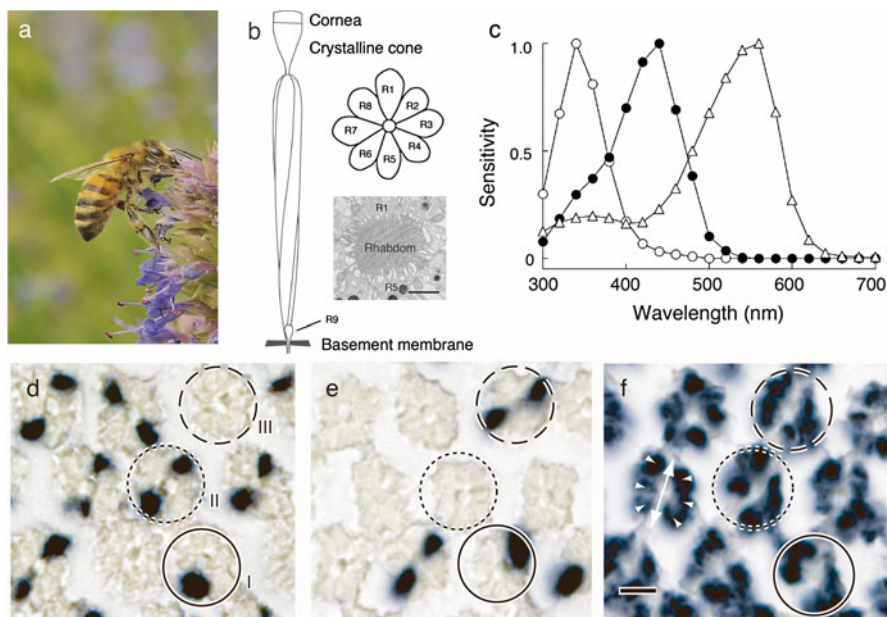


Fig. 5.3 Spectral receptors of honeybees. (a) The honeybee *Apis mellifera* (courtesy of Keram Pfeiffer). (b) Diagram of an ommatidium with the twisted photoreceptor bundle, and an electron micrograph of a transverse section of a rhabdom (bar: 1 μm). R1–R9, photoreceptor numbers. (c) Spectral sensitivities of UV, B and G photoreceptors. (d–f) Three consecutive transverse sections of the retinal layer, showing in situ hybridization of mRNAs encoding opsins of UV (d), blue (e), and green (f) absorbing visual pigments. The three types of ommatidia are indicated by *closed* (type I), *dotted* (type II), and *dashed* (type III) circles. The G probe (f) labeled six photoreceptors in all ommatidia (*white arrowheads*) (bar d–f: 5 μm)

The shape of a visual pigment's absorption spectrum is determined by the chromophore present but is otherwise generally well described by a template formula, with the peak wavelength value as the only variable (Govardovskii et al. 2000; Stavenga 2010). It thus was shown that AmUV is a visual pigment absorbing maximally at 353 nm (R353) and AmB is an R439 (Townson et al. 1998), matching the measured spectral sensitivities of the photoreceptor classes (Fig. 5.3c).

The question relating to which photoreceptors the individual visual pigments are expressed in was first studied histologically after monochromatic stimulation (Gribakin 1969), and then by a combination of single cell electrophysiology and intracellular dye injection (Menzel and Blakers 1976). These first results indicated that each ommatidium contains three UV (R1, R5, and R9), two B (R2, R6), and four G (R3,4,7,8) receptors. This view has been widely accepted, but a recent in situ hybridization study of opsin mRNAs in the retina indicated that some adjustments are necessary (Wakakuwa et al. 2005). The in situ hybridization revealed that the R1 and R5 photoreceptors are either UV or B receptors, while R2–4 and R6–9 are G receptors (Wakakuwa et al. 2005). A particularly interesting finding was that there are three ommatidial types. According to the mRNAs contained in R1 and R5, type I ommatidia have one UV and one B receptor, while type II and III have two UV and two B receptors, respectively: the ommatidia are themselves spectrally heterogeneous.

The three types of ommatidia are distributed somewhat randomly in an otherwise hexagonal pattern, but some regionalization exists. The dorsal region contains more type II ommatidia, with two UV receptors, while the ventral region contains more type III, with two B receptors (Wakakuwa et al. 2005). The concentration of B receptors in the ventral region of the eye is probably related to a better contrast detection by B receptors of terrestrial targets (Giurfa et al. 1999). Behavioral observations indicate that the dorsal rim area, which is crucial for polarization-based navigation, contains exclusively UV receptors (Helversen and Edrich 1974), but this has not yet been confirmed at the molecular level.

The retinal organization found in honeybees seems very similar to that of the bumblebee *Bombus impatiens* (Spaethe and Briscoe 2005). Its eyes are also furnished with a set of UV, B and G receptors (Skorupski and Chittka 2010). Peitsch et al. (1992) measured photoreceptor spectral sensitivities of 43 hymenopteran species from 14 families of bees and wasps, most of which appeared to have a set of three spectral receptors. In some species UV and/or B receptors were not found, but this may have been due to incomplete electrophysiological measurements (Peitsch et al. 1992). Four species were found to have photoreceptors with spectral sensitivities peaking at around 590–600 nm. The opsins expressed in these “red (R)” receptors have not yet been identified, but the unusual spectra may well be caused by spectral filtering (see below). The presently available data nevertheless clearly show that hymenopteran species share very similar sets of spectral photoreceptors.

The spectral sensitivities can be modified by the anatomical characteristics of the rhabdom. In the fused rhabdom of bees, the rhabdomeres of different classes of spectral receptors are tightly packed together (Fig. 5.3b). Because the fused rhabdom acts as an optical waveguide, the visual pigments in the various rhabdomeres forming the fused rhabdom mutually act as spectral filters. This effect is called lateral filtering (Snyder et al. 1973). For example, when the rhabdomeres of B and G

receptors coexist in the rhabdom, lateral filtering shifts the sensitivity peak of the B receptors hypsochromically (toward shorter wavelengths), while it shifts the peak of the G receptors bathochromically (toward longer wavelengths). Furthermore, when UV, B and G receptors coexist, the sensitivity profile of especially the B receptor becomes narrower. Another possible effect modifying spectral sensitivities is self-screening. This will occur in very long rhabdomeres or rhabdoms, because then the upper visual pigment layers act as spectral filters for the lower layers. Self-screening predicts broadening of the spectral sensitivity, but actual electrophysiological measurements do not provide clear examples.

5.5 Visual Pigments of Butterflies—The Exemplary Case: *Papilio xuthus*

Color vision of colorful butterflies has attracted researchers for quite some time (Ilse 1928; Swihart 1969; Bernard 1979). In recent years, several novel and important phenomena related to the mechanisms underlying photoreceptor spectral sensitivities as well as color vision in this group of insects have been revealed.

The most extensively studied species in this respect is the Japanese yellow swallowtail, *Papilio xuthus* (Papilionidae, Fig. 5.4a). The eye of *Papilio* is furnished with at least six classes of photoreceptors, peaking in the UV (360 nm), violet (V, 400 nm), B (460 nm), G (540 nm), and R (600 nm) wavelength regions, or having a broad-band (BB) sensitivity. The B and G receptor classes each have two subclasses: the spectral sensitivities of the B receptors can be narrow (nB) and broad (bB), while those of the G class can be double-peaked (dG) or single-peaked (sG) (Fig. 5.4c–e). On the other hand, *Papilio* eyes express only five visual pigment opsins: one UV-absorbing (PxUV, for *Papilio xuthus* UV), one B-absorbing (PxB), and three L-absorbing (PxL1, PxL2, and PxL3) (Fig. 5.1; Table in Fig. 5.4). The existence of multiple L opsins is due to gene duplication events (Briscoe 2000).

An ommatidium of *Papilio* contains nine photoreceptors, R1–9 (Fig. 5.4b). Unlike in bees, the rhabdom of *Papilio* has three tiers and is not twisted (Arikawa and Uchiyama 1996). The distal two-thirds of the rhabdom consist of the rhabdomeres of four distal photoreceptors, R1–4, while the proximal one-third is made up of the rhabdomeres of four proximal photoreceptors, R5–8. The basal photoreceptor, R9, forms a small, third tier at the base of the rhabdom. In transverse section, each rhabdom is surrounded by four clusters of red or yellow pigment. These perirhabdomal pigments form an absorbing layer around the rhabdom. They thus act as red or yellow filters for the boundary wave of light that propagates immediately outside the rhabdom. Interestingly, a subset of red-pigmented ommatidia distinctly fluoresces under UV excitation. The fluorescent pigment exists in the distal portion of the rhabdom and is most likely 3-hydroxy-retinol (Arikawa et al. 1999a). In summary, the compound eyes of *Papilio* comprise a collection of three types of ommatidia: I, red; II, red and fluorescent; and III, yellow (Table in Fig. 5.4).

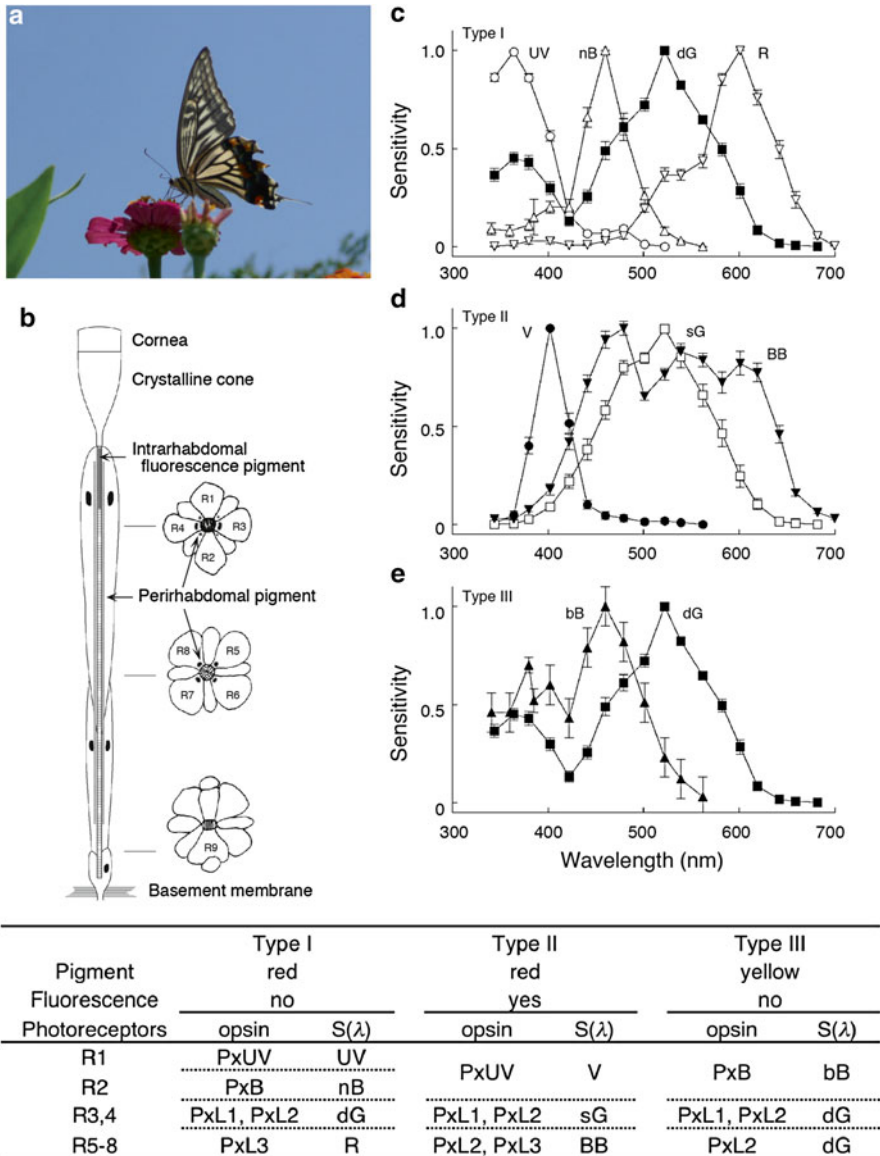


Fig. 5.4 Spectral organization of the retina of *Papilio xuthus*. (a) The Japanese yellow swallowtail, *Papilio xuthus*. (b) Schematic diagram of a *Papilio* ommatidium. The rhabdomeres of the R1–R9 photoreceptors form together a fused rhabdom, which is surrounded by clusters of red (type I and II ommatidia) or yellow (type III) perirhabdomal pigment. The rhabdom of type II ommatidia contains a fluorescent pigment. (c) Spectral sensitivities of the receptors of type I ommatidia. UV ultraviolet, nB narrow blue, dG double-peaked green, R red. (d) Spectral receptors in type II ommatidia. V violet, sG single-peaked green, BB broad-band. (e) Spectral receptors in type III ommatidia. bB broad blue. The table summarizes the spectral organization of the three types of ommatidia, with the localization of five opsins

Intracellular electrophysiology coupled with dye injection demonstrated that the location of all six classes of spectral receptors (eight when counting the B and G subclasses) could be unambiguously identified in the array of the three different types of ommatidia (Fig. 5.4c–e). This has now been combined with in situ hybridization studies of the five opsin mRNAs, to give a full understanding of the spectral organization of the *Papilio* eye (Arikawa 2003). R1 and R2 are either UV, V or B receptors, but their combination varies between the ommatidial types. Type I ommatidia have a UV and a narrow blue (nB) receptor, while type II have two violet (V) receptors and type III two broad-blue (bB) receptors. R3 and R4 are G receptors in all ommatidia (double-peaked green, dG, in type I and III ommatidia, and single-peaked green, sG, in type II). R5–8 are R receptors in type I, BB receptors in type II and dG receptors in type III ommatidia (Table in Fig. 5.4).

In general, individual photoreceptors express a single type of visual pigment, the so-called one rhodopsin per receptor rule (Stavenga and Arikawa 2008). Although the majority of photoreceptors follow this rule, it may not be universal, because opsin protein and/or its mRNA are found to be coexpressed in some photoreceptors of both invertebrates (Sakamoto et al. 1996; Rajkumar et al. 2010) and vertebrates (Roehlich et al. 1994; Makino and Dodd 1996; Lyubarsky et al. 1999; Applebury et al. 2000; Glosmann and Ahnelt 2002; Parry and Bowmaker 2002). An obvious question is whether these visual pigments participate in the phototransduction process. Some electrophysiological measurements to investigate this have been performed (Makino and Dodd 1996; Lyubarsky et al. 1999), but such attempts have remained rather rare. *Papilio* eyes have provided an early and conclusive proof. First it was reported that the R3 and R4 photoreceptors coexpress PxL1 and PxL2 mRNAs (Kitamoto et al. 1998). Because all R3 and R4 are G receptors throughout the eye, both PxL1 and L2 must be green-absorbing visual pigments. Furthermore, the R5–8 of type I ommatidia are R receptors and express PxL3 mRNA, indicating that the PxL3 visual pigment must be a red-absorbing visual pigment. Interestingly, the R5–8 of type II ommatidia coexpress PxL2 and PxL3 mRNA, and the spectral sensitivity of these photoreceptors is found to be broad, stretching from 400 to 650 nm (that is, a half-bandwidth of almost 210 nm compared with the usual 100 nm). The combined molecular and electrophysiological evidence clearly indicates that the PxL2 (green) and PxL3 (red) visual pigments are both functional in the broad-band (BB) receptors (Fig. 5.4d; Arikawa et al. 2003).

Papilio eyes yielded another unexpected result, namely that photoreceptors expressing the same opsin can have different spectral sensitivities. Both the UV receptors of type I ommatidia and the V receptors in the type II ommatidia express PxUV, which is a UV-absorbing visual pigment (Fig. 5.4d, Table in Fig. 5.4). The reason why PxUV-containing photoreceptors become V sensitive is the presence in type II ommatidia of a pigment, which acts as a UV-absorbing filter (Arikawa et al. 1999a). The perirhabdomal yellow and red pigments act also as spectral filters, but they only slightly shift the spectral sensitivities of the proximal R5–8 photoreceptors (Arikawa et al. 1999b).

5.6 Other Papilionid Species

As Briscoe et al. (2003) stated, “not all butterfly eyes are created equal.” This is in fact true even within the same family. The Glacial Apollo, *Parnassius glacialis*, is a member of the subfamily Parnassiinae in the family Papilionidae (<http://tolweb.org/Papilionidae/12177>). Their eyes express four visual pigments: one UV (PgUV, *Parnassius glacialis* UV), one B (PGB), and two L (PgL2 and L3). This indicates that in *Parnassius*, L opsin duplication happened only once, whereas in *Papilio*, a member of the tribe Papilionini, duplication must have happened twice (Fig. 5.1). The expression pattern of opsin mRNA is also quite different: in *Parnassius* type II and III, PgUV and PGB are coexpressed in R1 and R2. The R3 and R4 photoreceptors of type I and II express PgL2, presumably a green-absorbing pigment, while the R3 and R4 of type III express PgL3, which is probably red-absorbing (Matsushita et al. 2012).

A survey of opsin mRNAs in four tribes (Zerynthini, Troidini, Luehdorfini, and Leptocircini) in the subfamily Papilioninae revealed that all of the tested species have either two (Zerynthini and Troidini) or three (Luehdorfini and Leptocircini) L opsins, one B opsin, and one UV opsin. Evidently, in the lineage of Papilionidae, duplication of L opsins has occurred repeatedly, probably to acquire red receptors. Duplication appears to be absent in UV and B opsin clades, but nevertheless polymorphic short-wavelength receptors are present. This occurred in type II ommatidia of *Papilio* by the acquisition of a UV-absorbing, fluorescent pigment. Fluorescing ommatidia have been found in all papilionid species tested, indicating that the mechanism exists universally in papilionids (Matsushita et al. 2012; Chen et al. 2013). The eyes of the Common Bluebottle, *Graphium sarpedon* (Leptocircini), even show a further diversification: their ommatidia are either strongly, weakly or non-fluorescent. Because of this variation, they have at least four subclasses of B receptors (unpublished observation).

5.7 *Pieris rapae*: Effect of Perirhabdomal Filters

Although the optical filter effect of the red and yellow pigments is minor in Papilionidae, it is particularly strong in Pieridae (Fig. 5.5). The cellular arrangement of the ommatidia of the Small White butterfly, *Pieris rapae crucivora*, is similar to that of *Papilio*. In both cases, nine photoreceptors construct a tiered rhabdom: four distal (R1–4), four proximal (R5–8), and one basal (R9). However, in *Pieris* the trachea creates a tapetum proximal to each rhabdom. Light entering the facet propagates along the rhabdom until it is absorbed by the visual pigments or the perirhabdomal pigments. However, part of the light reaches the proximal end of the rhabdom without having been absorbed and is then reflected by the tapetum back into the rhabdom. A minor fraction of light escapes absorption even during the second trip and thus leaves the eye. This can be observed with epi-illumination microscopy and

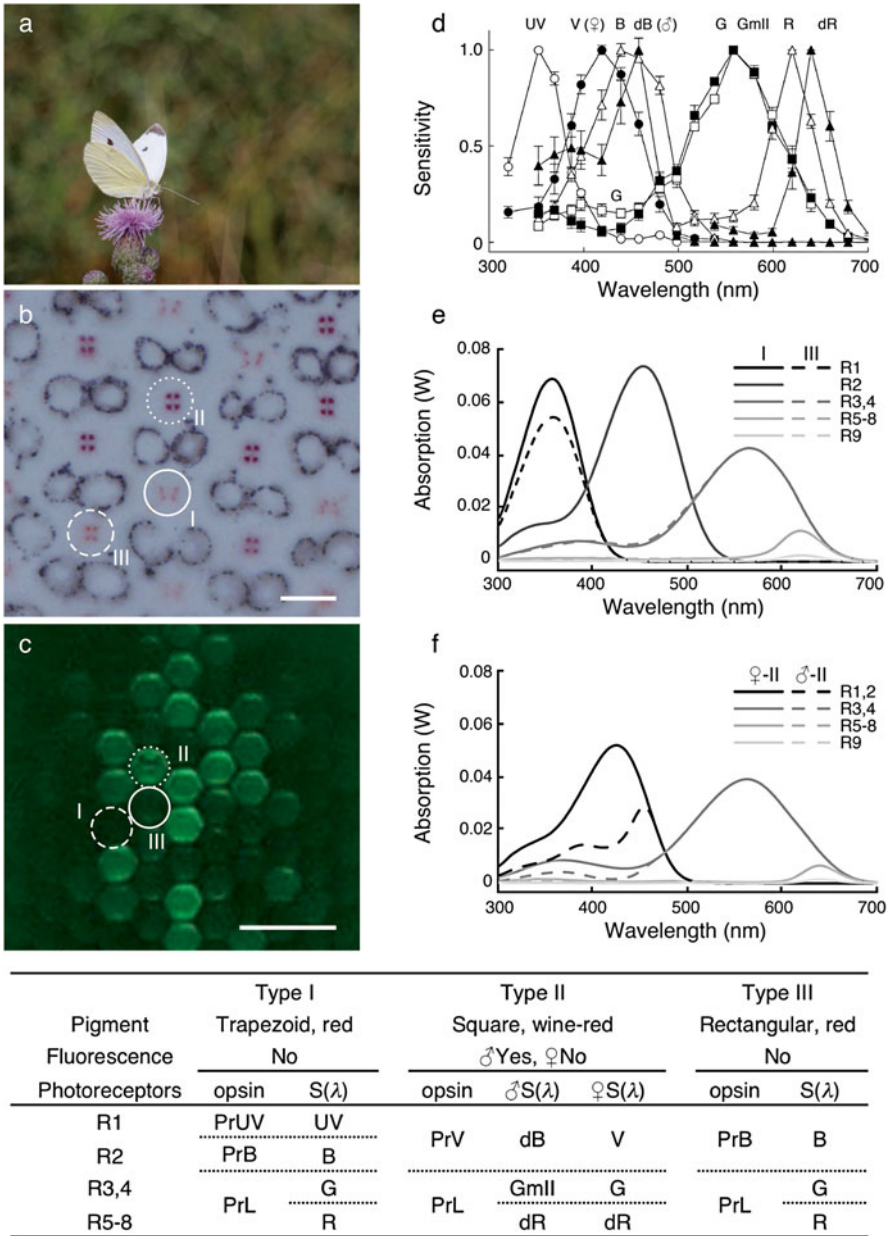


Fig. 5.5 Spectral organization of the retina of *Pieris rapae*. (a) Small white, *Pieris rapae*. (b) Unstained transverse section of an eye of *Pieris*. The color and arrangement of the perirhabdomal pigment identify the ommatidial types I, II and III; bar: 10 μ m. (c) Violet-induced green fluorescence of the same eye region shown in (b). Type II ommatidia are strongly fluorescent; bar: 50 μ m. (d) Spectral sensitivity functions recorded from single photoreceptors of *Pieris rapae*. UV ultraviolet; V violet, in female type II; B blue; dB double-peaked blue; G green; GmII green in male type II; R red; dR dark red. (e, f) Absorption spectra of R1–9 photoreceptors calculated with a wave-optics model for type I and III ommatidia (e) and the sexually dimorphic type II ommatidia (f) (Stavenga and Arikawa 2011). The table summarizes the spectral organization of the three types of ommatidia, with the localization of the four opsins

is called “eyeshine.” The eyeshine color depends on the light absorption and tapetal reflection of the ommatidial types. In the ventral two-thirds of the *Pieris* eye, the rhabdoms are surrounded by either orange-red or wine-red colored screening pigments, so that with white-light illumination, a red or dark-red colored eyeshine results, with reflectance spectra of the different ommatidia peaking at 635 or 675 nm (Qiu et al. 2002).

The color and the spatial arrangement of the perirhabdomal pigments demonstrate that the eyes of *Pieris* consist of three ommatidial types. Transverse sections show that in all types, four clusters of pigment surround the rhabdom. The pigment clusters are arranged in a trapezoidal (type I), square (type II), or rectangular (type III) pattern (Fig. 5.5b). Type I and III ommatidia contain the orange-red pigment, while in type II the pigment is wine-red. In addition, the distal part of the rhabdom of type II ommatidia contains a fluorescent pigment that functions as an optical filter (green emission under violet excitation, Fig. 5.5c), but the fluorescent pigment exists only in males. The eyes of *Pieris* thus are sexually dimorphic (Arikawa et al. 2005).

The eyes of *Pieris* express four opsins, which belong to UV- (PrUV, *Pieris rapae* UV), violet- (PrV), blue- (PrB) and long wavelength-absorbing (PrL) classes (Fig. 5.1). Unlike in *Papilio*, a gene duplication appears not to have happened in *Pieris* in the L opsin clade, but rather in the M opsin clade, creating distinct B- and V-opsins. In situ hybridization revealed that although the distal R1 and R2 photoreceptors express one visual pigment, they do so in three combinations: PrUV in R1 and PrB in R2, or vice versa (type I), PrV in both R1 and R2 (type II), or PrUV in both R1 and R2 (type III). The distal R3–4 and the proximal R5–8 all express the PrL opsin (Wakakuwa et al. 2005).

The spectral sensitivities of the distal R1 and R2 photoreceptors are rather simple, because the PrUV- and PrB-expressing photoreceptors straightforwardly correspond to UV and B receptors. The PrV-expressing receptors in type II ommatidia have a peak sensitivity at 420 nm in females, but those of males are maximally sensitive at 460 nm. The latter appears to be caused by a fluorescent pigment in the type II ommatidia of males, which acts as a spectral filter. The consequence of the sexual dimorphism of the filter pigment thus is that it produces sexually dimorphic spectral sensitivities (Fig. 5.5d, Table in Fig. 5.5).

In order to address the question how the duplicated opsins have acquired different spectral absorption spectra, accurate spectroscopic analyses are required. In vitro expression of invertebrate visual pigments in cultured cells has been successful only in a few cases (Terakita et al. 2008; Nagata et al. 2012) including *Pieris rapae* (Wakakuwa et al. 2010). In vitro reconstitution of the duplicated opsins PrB and PrV has revealed that the absorption spectra of these pigments peak at 450 nm and 420 nm, respectively. Among 24 amino acid residues located within 5 Å from any carbon of the chromophore, the amino acids at positions 116 and 177 were found to be crucial for the spectral tuning (the numbering according to squid rhodopsin). The amino acids of these sites are Ser (116) and Phe (177) in PrB, while they are Ala and Tyr in PrV. Because most lepidopteran B opsins so far identified have Ser and Phe at these sites, which is also the case in PrB, the amino acid pair

(Ser/Phe) must be ancestral in the B clade. Substituting Ser116 by Ala in PrB resulted in a 13 nm short-wavelength shift, from 450 to 437 nm, and substituting Phe117 by Tyr resulted in a 4 nm short-wavelength shift, to 446 nm in the mutant molecule. This tuning mechanism appears to be shared, at least in part, by the pigments of other pierid and lycaenid butterfly species (Wakakuwa et al. 2010).

The spectral sensitivities of the PrL-expressing photoreceptors cannot be understood without considering the contribution of the perirhabdomal pigments (Fig. 5.5b). In the distal tier, where the effect of the pigments is negligible, the spectral sensitivity of the PrL-containing R3 and R4 photoreceptors well match the absorption spectrum of a visual pigment maximally absorbing at 563 nm, indicating that the PrL is an R563 pigment. In the proximal tier, the spectral sensitivity of R5–8 in type I and III ommatidia, where the perirhabdomal pigment appears orange-red, peaks at 620 nm (R receptor). The sensitivity of the R5–8 in type II ommatidia with wine-red pigment peaks at 640 nm (DR receptor). Evidently, the large sensitivity shifts, from 563 to 620 and 640 nm, are caused by the orange-red and wine-red pigments acting as spectral filters (Wakakuwa et al. 2004).

It should be noted that the above descriptions only hold for the ventral two-thirds of the *Pieris* eye. The situation is somewhat different in the dorsal one-third, because the *Pieris* eye exhibits a distinct degree of regionalization, a phenomenon generally encountered in compound eyes. Regionalization can be directly observed in butterfly eyes with tapeta, since the ventral and dorsal eye often exhibit quite different eyeshines (Stavenga et al. 2001). Whereas the eyeshine is reddish ventrally, it is yellow in the dorsal region of the *Pieris* eye. The latter is due to the absence of perirhabdomal pigments. The dorsal region also contains three types of ommatidia, but the R1 and R2 of the type II ommatidia in the dorsal region express PrB but not PrV. Close examination of the eyeshine in *Pieris rapae* with monochromatic light has revealed a transitional zone with six rows of ommatidia between the ventral and dorsal regions. In the transitional zone, these photoreceptors coexpress both PrB and PrV. The coexpression may be due to the overlapping of regulatory factors determining the ventral and dorsal regions during the developmental process.

The multitude of visual and screening pigments, together with sexual dimorphism and regionalization, makes the *Pieris* eye extremely complex. Nevertheless, the combination of anatomy, intracellular electrophysiology, in situ hybridization, microspectrophotometry, and optical observations using the eyeshine effect has produced a sufficiently comprehensive knowledge to allow quantitative optical modeling of the spectral sensitivities of all photoreceptors (Stavenga and Arikawa 2011). Specifically, the modeling revealed that the absolute sensitivities of the proximal R5–8 photoreceptors are significantly reduced due to the strong screening effect of the perirhabdomal pigments (Fig. 5.5e, f) (Stavenga and Arikawa 2011). The modeling indicated that the small basal photoreceptor, R9, for it to have any functional light sensitivity, must express PrL. Even then the light sensitivity is very low. Presumably therefore, PrL functions specifically in bright light conditions.

5.8 *Colias erate*, Sexual Dimorphism

Sexual dimorphism of the retina is even more pronounced in the Eastern Clouded yellow butterfly, *Colias erate*, a member of the subfamily Coliadinae in the family Pieridae. The wings of males are yellow, but most females are white; some females (less than 15 %) have a male-like yellowish color (Watanabe and Nakanishi 1996). The larvae of this species feed on clover leaves, and mated females select high quality leaves to lay eggs on. As in *Pieris rapae*, the retina of *Colias* is composed of three types of ommatidia with the perirhabdomal pigments arranged trapezoidally (type I), square (type II), and rectangular (type III) (Fig. 5.6b). Although belonging to the same family, it differs from *Pieris* in that both sexes have fluorescing ommatidia, namely the type I ommatidia in males and type II in females. In addition, the color of the perirhabdomal pigment in female type II ommatidia is paler. These rather subtle differences contribute to a clear sexual dimorphism (Ogawa et al. 2012, 2013).

As shown in Fig. 5.1, the eyes of *Colias erate* express five visual pigment opsins: CeUV (*Colias erate* UV), CeB, CeV1, CeV2, and CeL (Awata et al. 2009; Ogawa et al. 2012). The expression pattern of the mRNAs is summarized in Fig. 5.6. R1 and R2 express S (CeUV) and M (CeB, CeV1, and CeV2) opsins, while the R3–8 photoreceptors express the L opsin (CeL), as in other species. The most conspicuous feature here is the colocalization of M opsins. The mRNAs of CeV1 and CeV2 are always colocalized, but in type II ommatidia of the ventral region they are coexpressed together with CeB. The UV opsin, CeUV, is expressed in type I and III ommatidia. No sexual dimorphism has been detected in the opsin expression pattern (Fig. 5.6, Table).

The reason why CeV1 and CeV2 are always expressed together is not known, but presumably the genes are localized in tandem downstream of a common promoter. At the present stage, the two visual pigments have clearly not yet been subjected to subfunctionalization. In fact, the spectral sensitivity of photoreceptors expressing both CeV1 and CeV2 in non-fluorescing ommatidia (female type I, Fig. 5.6f) closely matches the absorption spectrum of an R430 pigment, suggesting that the absorption spectra of CeV1 and CeV2 are very similar. The corresponding photoreceptors in males exist in fluorescing ommatidia and have a narrower spectral sensitivity peaking at 440 nm, with a kink at 420 nm (Fig. 5.7c). The spectral shift is evidently caused by the fluorescent pigment acting as a spectral filter.

The situation in type II ommatidia is reversed, because in these ommatidia, the fluorescent pigment exists only in females. The R1 and R2 photoreceptors in type II ommatidia of both males and females express three M opsins, CeB, CeV1 and CeV2. In males, these photoreceptors have a broad spectral sensitivity, with half bandwidth about 150 nm and peak wavelength 460 nm (Fig. 5.7d). The bB spectral sensitivity can be understood from the colocalized visual pigments R430 (CeV1 plus CeV2) and R460 (CeB) (Ogawa et al. 2012). In females, the R1 and R2 photoreceptors of type II ommatidia become nB receptors, peaking at 460 nm (Fig. 5.7g), due to the presence of a fluorescent pigment acting as a spectral filter.

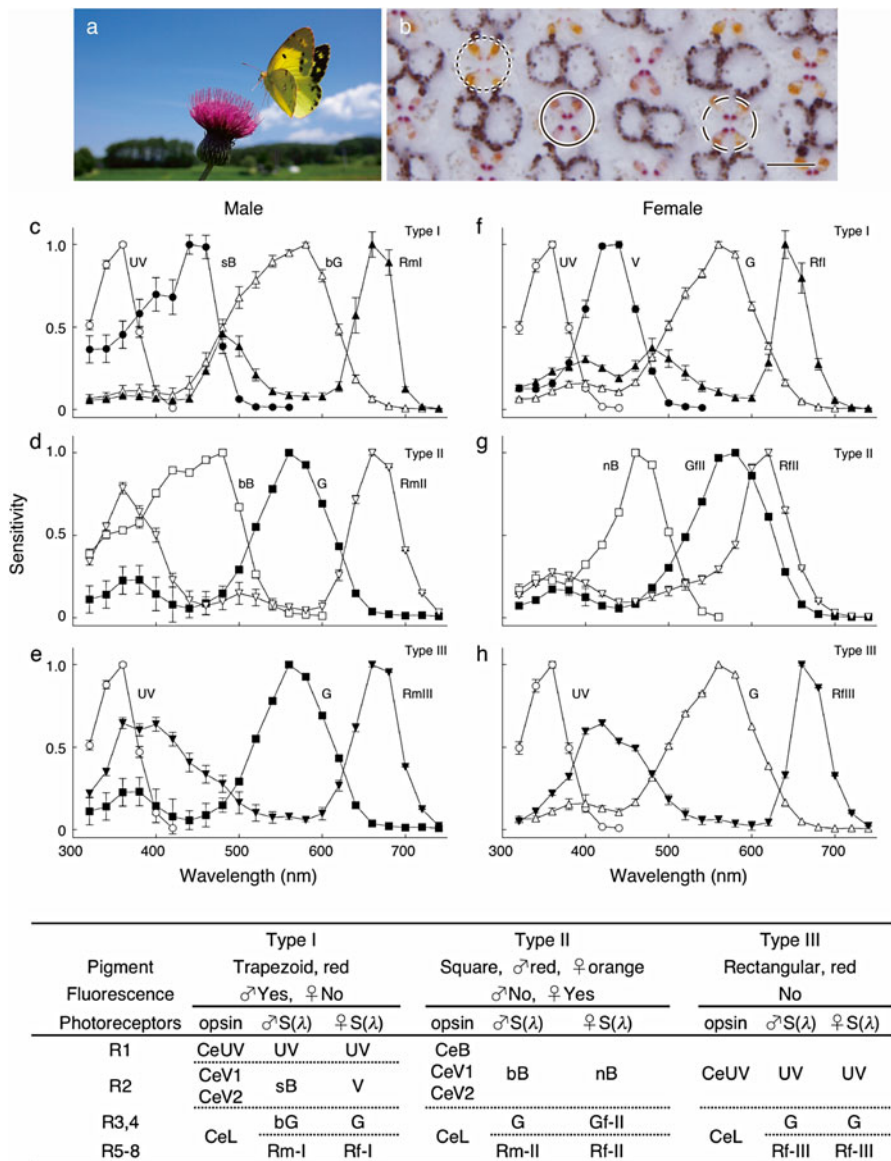


Fig. 5.6 Spectral organization of the retina of *Colias erate*. (a) A male Eastern clouded yellow, *Colias erate* (courtesy of Kazuo Unno). (b) Unstained transverse section of the female retina. Three types of ommatidia are indicated by closed (type I), dotted (type II), and dashed (type III) circles (bar: 10 μm). (c) Spectral receptors in male type I ommatidia. UV ultraviolet, sB sharp blue, bG broad green, Rml red of male type I. (d) Male type II. bB broad blue, G green, RmII red of male type II. (e) Male type III. RmIII red of male type III. (f) Female type I. V violet, RfI red of female type I. (g) Female type II. nB narrow blue, GfII green of female type II, RfII red of female type II. (h) Female type III. RfIII red of female type III. The table summarizes the spectral organization of three types of ommatidium, with the localization of five opsins

The most prominent sexual dimorphism is found in the long wavelength receptors in *Colias*, which all express CeL. In the distal tier, the CeL-expressing R3 and R4 photoreceptors are all green-sensitive, with peak sensitivity at 560 nm. The spectral sensitivity can be reasonably well explained with the spectrum expected for an R565 visual pigment (Ogawa et al. 2012). In the proximal tier, however, the R5–8 of all ommatidial types in males are red-sensitive peaking at 660 nm (Fig. 5.7c–e). This shift of about 100 nm is readily explained by the filtering effect of the perirhabdomal red pigment. The red filter effect is probably enhanced by the strong constriction of the rhabdoms occurring between the distal and proximal tiers (Arikawa et al. 2009). In females, the spectral sensitivity of the R5–8 differs among the ommatidial types. They peak at either 650 (type I ommatidia), 610 (type II), or 660 nm (type III). The differences in the sensitivity peak shifts, from 565 to 650, 610 and 660 nm, respectively, is due to the differences in the spectral and spatial properties of the screening pigments (Fig. 5.7f–h). Notably the type II ommatidia of females contain a pale-orange screening pigment, which causes a much smaller spectral shift than the red pigment in the other ommatidial types (Ogawa et al. 2013).

A set of photoreceptors with different spectral sensitivities provides an animal with the ability to discriminate light of different wavelengths (von Helversen 1972; Koshitaka et al. 2008). Pierid butterflies appear to have an amazingly pronounced sexual dimorphism in the spectral sensitivities of their photoreceptors. Most likely, the male and female butterflies view the colored world quite differently, especially in the red wavelength region. This ability may be crucial for females to judge the quality of leaves on which to lay eggs (Ogawa et al. 2013).

5.9 *Lycaenidae*, Sexual Dimorphism in Opsin Expression

The expression pattern of the visual pigment opsins in Pieridae is identical in both sexes, and hence the sexual dimorphism in the photoreceptor spectral sensitivities is due to differences in screening pigments. *Lycaenidae* appear to have followed another strategy to create sexual dimorphism in the photoreceptor spectral sensitivities by changing the opsin expression pattern between sexes: males and females express opsins differently (Sison-Mangus et al. 2006).

As in Pieridae, several species of *Lycaenidae* have duplicated M opsins, in addition to one S and one L opsin (Fig. 5.1). In the Ruddy Copper butterfly, *Lycaena rubidus*, the absorption spectra of their UVRh, BRh1, BRh2, and LWRh (Sison-Mangus et al. 2006) have peak wavelengths at 360 nm, 437 nm, 500 nm and 568 nm, respectively. The UVRh, BRh1 and BRh2 opsins are complementarily expressed in R1 and R2 photoreceptors in six fixed combinations (UV-UV, B1-B1, B2-B2, UV-B1, UV-B2, B1-B2) throughout the eye, while other butterfly species typically have three ommatidial types. This spectral variety in the eyes of *Lycaena rubidus* suggests that, with appropriate neuronal wiring, they may have a better spectral resolution, particularly in the blue wavelength region (Sison-Mangus et al. 2006). The possible enhancement of color vision in the blue region has been considered to

be useful for conspecific visual communication in these blue butterflies (Sison-Mangus et al. 2006). Upon searching for flowers that provide nectar, the duplicated B opsins appear also to be useful to discriminate greenish colors in other lycaenid species, *Polyommatus icarus* (Sison-Mangus et al. 2008). However, electrophysiological evidence demonstrating that the various photoreceptor classes have different spectral sensitivities is not yet available.

Sexual dimorphism is observed in photoreceptors R3–8 of Lycaenidae. In the ventral region of the eye these photoreceptors all express the L opsin. In the dorsal region of male eyes, R3–8 exclusively express the B1 opsin mRNA, while in females the R3–8 coexpress the B1 and L opsin mRNAs (Sison-Mangus et al. 2006). The latter photoreceptors most likely therefore have an extremely broad spectral sensitivity, as in the *Papilio* BB receptors that coexpress the green-absorbing PxL2 and red-absorbing PxL3 (Fig. 5.4).

5.10 Dipterans: *Drosophila* and Other Flies

A considerable part of our present understanding of insect vision has been gained by research on the visual systems of flies, that is, the higher Diptera. Specifically the fruitfly, *Drosophila melanogaster*, has played a key role in the unraveling of the molecular properties of insect visual pigments, because this is one of the most important model organisms in biology and most of the modern genetic and molecular tools are available in this species (Fig. 5.7). The compound eye of *Drosophila* consists of about 800 ommatidia, each containing eight photoreceptors, R1–8. The rhabdomeres of R1–6 are spatially separate and surround the rhabdomeres from R7 and R8, which are positioned in tandem (Fig. 5.7a). The crystalline anatomy of fly eyes has been useful for unraveling retinal properties, but this has been supported and extended by the existence of many relevant gene mutations.

The complete set of six visual pigments of *Drosophila* (Rh1–Rh6) was identified by measuring difference spectra of retinal extracts in two extreme photosteady states (Salcedo et al. 1999) (Fig. 5.7b). An analysis of the difference spectra with visual pigment template formulae (Stavenga et al. 1993) yielded the rhodopsin and metarhodopsin spectra for each visual pigment type. The derived peak wavelengths are given in Fig. 5.7b; e.g. the rhodopsin of Rh1, R486, absorbs maximally at 486 nm and its metarhodopsin, M566, at 566 nm. The amplitude of the absorbance coefficient of the metarhodopsins relative to that of their rhodopsin varies between 1.4 and 1.7.

The blue–green absorbing Rh1 visual pigment is present in all R1–6 photoreceptors. The blue-absorbing Rh2 was found to be the visual pigment of the ocelli. The exclusively UV-absorbing Rh3 occurs in the R7 photoreceptors of 30 % of the ommatidia, randomly distributed throughout the eye. These ommatidia are called p-type, based on their similarity to the UV-absorbing rhodopsin in the R7 of the p-type ommatidia of larger flies (Hardie 1985). Rh4, which also absorbs UV, is found in the complementary y-type ommatidia, which makes up the remaining 70 % of R7 photoreceptors. The blue-absorbing Rh5 opsin is expressed in all R8s of the p-type

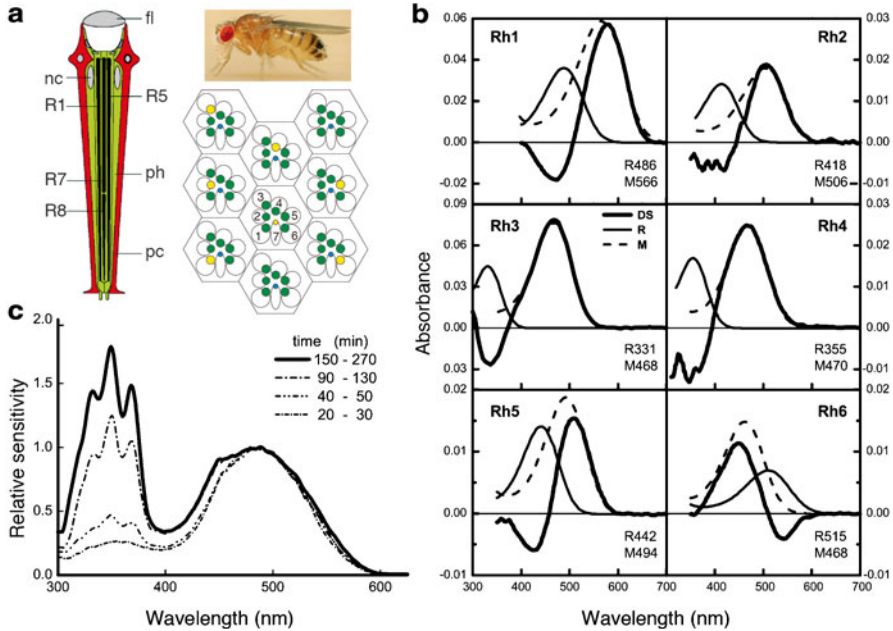


Fig. 5.7 *Drosophila* and blowfly. **(a)** The wild type fruitfly eye is red due to pigments in the cells that surround the pseudocone, the structure proximal to the facet lens (fl). The red pigment optically isolates the ommatidia from each other. Each fly ommatidium contains eight photoreceptor cells, R1–8. The six large, outer or peripheral photoreceptors, R1–6, have long and fat rhabdomeres, and the two slender, inner or central photoreceptors, R7 and R8, have thinner rhabdomeres, arranged in tandem, with R7 distal and R8 proximal. The photoreceptor cells (ph), with distal nucleus (nc), are surrounded by screening pigment cells (pc). The cross-section diagram shows that the rhabdomeres of R1–6 (green) have a characteristic trapezoidal pattern, with central the R7,8 rhabdomeres. The light-colored rhabdomeres mark photoreceptors that share the same visual direction and that project their axons onto the same higher order neurons. **(b)** Spectral characteristics of the visual pigments Rh1–Rh6 of the fruitfly *Drosophila*. Difference spectra (DS) were measured from eye extracts and fitted with calculated absorbance spectra of visual pigments using template formulae. The peak wavelengths (in nm) of the concluded rhodopsin (R) and metarhodopsin (M) spectra are indicated by the numbers (modified from Salcedo et al. 1999). **(c)** Incorporation of a sensitizing pigment in the photoreceptors of vitamin-A-deprived blowflies, resulting in an enhanced sensitivity in the UV with respect to that in the blue–green. The enhancement is due to binding of a 3-hydroxy-retinol to rhodopsin. UV-light absorbed by the 3-hydroxy-retinol then results in transfer of energy to the chromophore of rhodopsin, 3-hydroxy-retinol. The fine structure emerging in the UV is interpreted to be caused by a rigid binding of the 3-hydroxy-retinol. The spectra were measured at the indicated time after supplying all-*trans* retinal to the eye (modified from Hamdorf et al. 1992)

ommatidia; the green-absorbing Rh6 opsin is expressed in the R8s of the y-type ommatidia. The 70 % y-type ommatidia are non-homogeneous, with 60 % expressing only Rh4, while 10 % coexpress Rh3 and Rh4; the latter localize in the dorsal third of the *Drosophila* eye (Mazzoni et al. 2008). As the double expression of the UV rhodopsins occurs in the R7s in the dorsal third of the compound eye, Mazzoni et al. (2008) hypothesized that these R7 photoreceptors, together with the underlying

R8s, function in analyzing the UV light in the sky, specifically to detect differences in the solar and non-solar parts of the sky—that is, sky near the sun and away from it—which can differ considerably in short-wavelength light content. This skylight-discriminating ability may serve to help the fly orient for navigational purposes.

The absorption spectrum of visual pigments generally consist of different absorption bands that are called α , β , γ , etc. (Stavenga and van Barneveld 1975). Figure 5.7b shows the α -bands of the fruitfly visual pigment states, rhodopsin and metarhodopsin, but the β -bands were not determined. For all visual pigments studied in extracts, the amplitude of the β -band is much smaller than the amplitude of the α -band and restricted to the UV wavelength range. Surprisingly, early electrophysiological studies of R1–6 fly photoreceptors commonly yielded spectral sensitivities in the UV that were as large as the sensitivity at around 500 nm, the presumed α -band range (Hardie 1979). Extensive research by Kirschfeld and co-workers has revealed that the high UV-sensitivity is created by a 3-hydroxy-retinol molecule that is additionally bound to the opsin and acts as a sensitizing pigment (Kirschfeld et al. 1977). The 3-hydroxy-retinol absorbs UV light and transfers the energy to the native chromophore, 11-*cis* 3-hydroxy-retinal, resulting in activation of the rhodopsin molecule. The multiple peaks in the UV wavelength region in the spectral sensitivity (Fig. 5.7c) are attributed to the absorption spectrum of the 3-hydroxy-retinol molecule.

The action of the sensitizing pigment was directly demonstrated in a series of experiments in the blowfly *Calliphora vicina* reared on different vitamin-A diets (Hamdorf et al. 1992). Photoreceptors of blowflies reared on a vitamin-A-deprived diet have a low absolute light sensitivity, due to the necessity of vitamin A for producing rhodopsin. Supplying retinoids results in an increased visual pigment concentration, as witnessed by an increased sensitivity. Figure 5.7c presents the sensitivity spectra, normalized to the sensitivity peak of the α -band, measured after application of all-*trans*-retinal to the eye. The initial spectral sensitivity closely resembles a classical rhodopsin spectrum, with a low sensitivity band in the UV. Within a few hours this band had risen considerably and then featured a prominent vibronic fine structure, with peaks at 333, 350 and 369 nm. These peaks prove the presence of 3-hydroxy-retinol. Evidently, this derivative was enzymatically produced from the administered all-*trans*-retinal.

5.10.1 Spectral Characteristics of Insect Visual Pigments

Figure 5.7b shows that in addition to the difference in absorbance, the peak wavelengths of the two photostable visual pigment states often differ considerably. Interestingly, the absorption peak wavelengths of rhodopsin and metarhodopsin have characteristic relationships for the visual pigments of *Drosophila* as well as other insects (Fig. 5.8). The peak shift of the S and M (UV and B) visual pigments is always bathochromic, while for the L (G) visual pigments the peak shift is hypsochromic. The spectral shift for the S visual pigment is positive and generally large (about 130 nm). For the M-opsins, the shift is much smaller (50–80 nm), whereas

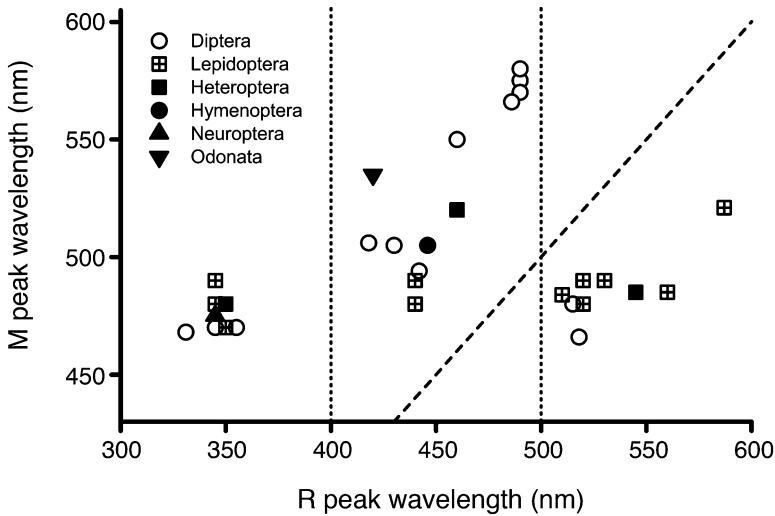


Fig. 5.8 The absorption peak wavelength of metarhodopsin as a function of the absorption peak wavelength of its rhodopsin for a number of insect orders. The three visual pigment types, UV-, blue, and long wavelength-absorbing are roughly separated by the *dotted vertical lines*. At the oblique *dashed line* the peak wavelengths of rhodopsin and metarhodopsin are identical. The S-(UV-) and M-(blue) rhodopsins have bathochromic-shifted metarhodopsins, but the L-(long-wavelength-absorbing) rhodopsins have a hypsochromic-shifted metarhodopsin

the spectral shift for the L-opsins is negative, ~ 40 nm. No interpretation in molecular terms has yet been formulated, but the differences in spectral shift have important consequences for the regeneration processes of the visual pigments in the eyes of different insect species (Stavenga 1992).

An important consequence of the bistability of insect visual pigments is that under bright daylight conditions, every visual pigment molecule of an insect eye regularly shuttles back and forth from the rhodopsin to the metarhodopsin state. When unprotected by a light-controlling pupil mechanism, the conversion rates in fly photoreceptors in natural light conditions are of the order of seconds (Stavenga and Hardie 2011), but with a closed pupil each visual pigment molecules will flip-flop about once in every minute.

Fly eyes contain, like butterflies, red screening pigments, but their location and function is very different. The red screening pigments are not located in the photoreceptors, but in separate screening pigment cells (Fig. 5.7a). These cells surround the photoreceptors and thus protect them for activation by off-axis stray light. Yet, with incident broad-band white light, the high transmittance of the screening pigment cells in the longer wavelength range results in a considerable amount of red stray light, which evades the photoreceptor layer. This is unimportant for the R486 pigment that is concentrated in the rhabdomeres of the R1–6 photoreceptors, because it predominately absorbs only at rather short wavelengths. However its metarhodopsin form, M566, readily absorbs red light, and thus the red stray light

can favorably convert the metarhodopsin into its rhodopsin state. The function of the red-transmittant screening pigment cells is thus to support photoregeneration of the visual pigment (Stavenga and Hardie 2011).

This elegant method of visual pigment regeneration does not work for green-absorbing rhodopsins, because red stray light will be preferably absorbed by the rhodopsin molecules where it will cause unwanted background noise. Most insect eyes therefore have strongly absorbing, brown–black screening pigments because the majority of their photoreceptors rely on green-absorbing rhodopsins, as shown above for the cases of bees and butterflies (see Figs. 5.5b and 5.6b). The regeneration of rhodopsin from metarhodopsin then must occur through a complicated, enzymatic renewal cycle, involving the degradation of metarhodopsin and renewal of rhodopsin, requiring numerous cellular components of the retina (Schwemer 1984, 1989; Smith and Goldsmith 1991; Wang et al. 2010). The speed of decay is faster than that of the regeneration, and both strongly depend on temperature (Bernard 1983). Under bright light conditions, the green-absorbing visual pigments are “bleached,” that is, their concentration is reduced. Interestingly, this expands the intensity range where the photoreceptors can function, just as occurs with human cone photoreceptors (Stavenga and Hardie 2011).

5.11 Concluding Remark

Insects are particularly interesting for studying the evolution of visual pigments because of their phylogenetic variety, different lifestyles (diurnal vs. nocturnal) as well as the variety of compound eye structures (apposition vs. superposition). Here, we focused on the mechanisms underlying the spectral sensitivities of insect photoreceptors. The main player is of course the visual pigment. In order to produce photoreceptors of different spectral sensitivities, duplication of opsin genes is therefore the most straightforward mechanism. In fact, opsin duplication appears to happen repeatedly in a variety of animal lineages. Duplicated visual pigments are sometimes coexpressed in single photoreceptors, making their spectral sensitivities abnormally broad when the absorption spectra of visual pigments have diverged.

In addition to the visual pigments, various other photostable pigments act as spectral filters that significantly modify the absorption spectra of the visual pigments *in situ*, and thus enact a crucial function for the final shaping of the spectral sensitivities. The photostable pigments include the sensitizing pigment and the perirhabdomal and intrarhabdomal (fluorescent) filter pigments. The sensitizing pigment is found only in higher flies, but filter pigments are widely encountered among various insects, including butterflies and hymenopterans, and even can produce sexual dimorphism in the spectral sensitivities of the photoreceptors.

Information about spectral tuning at the level of opsin molecules is rather sparse in insects. This is because any stable technique for expressing insect visual pigments *in vitro* is lacking. Some pigments in honeybees (Terakita et al. 2008), the small white butterfly (Wakakuwa et al. 2010), and the Adanson jumping spider (Nagata et al. 2012) have been expressed in human embryonic kidney (HEK) cells.

However these examples are exceptional, and it has been still quite difficult to express and reconstruct insect rhodopsins *in vitro*. Overcoming this technical barrier will considerably stimulate the study of insect rhodopsins and will possibly uncover the evolutionary background of their amazing adaptation to a variety of light environments.

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