# 1

# Invertebrate photoreceptor optics

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## 1.1 INTRODUCTION

Animals sustain their life by accumulating the necessary energy from their environment, requiring the continuous acquisition of information with their sensory systems. The prime sensory instrument of many animals is the visual system, with the photoreceptors in the retina as the main elements sampling the visual information. To gain insight into the spatial and spectral properties of the photoreceptors, it is essential to understand the optics of the eyes. The optics of invertebrate eyes and their photoreceptors, the theme of this chapter, has been described in many excellent reviews published in the recent decades (Snyder and Menzel, 1975; Miller, 1979; Snyder, 1979; Land, 1981; Nilsson, 1989; Warrant and McIntyre, 1993; Land and Nilsson, 2002). Here, we discuss a number of recent developments.

Vision starts when a visual pigment molecule absorbs a photon. The absorbed light energy excites the molecule, which then goes through a series of photochemical steps and ultimately causes phototransduction, that is, triggers the chain of molecular processes that results in a neural signal (see Chapter 2). The visual pigment molecules are embedded in a specialised organelle of the photoreceptors that is constructed from an intricately folded part of the cell membrane. Accordingly two main types of photoreceptors can be distinguished, ciliary and rhabdomeric photoreceptors, depending on the cellular origin of the visual pigment-bearing organelle: a cilium or a stack of microvilli, respectively (Eakin, 1972; Arendt *et al.*, 2004). This structure often adopts a cylindrical shape and thus acts as an optical waveguide, since the material within the cylinder, with a high concentration of cell membranes composed of phospholipids

and proteins, has a higher refractive index than the watery surroundings.

The way visual pigments are packed into an optical waveguide has important functional consequences. Light that enters the eye and is channelled into the waveguide has an enhanced chance of being captured by the visual pigment molecules. This light channelling is restricted to paraxial directions, and therefore the waveguide properties confer directional sensitivity on the photoreceptive element. A lens, a light-funnelling cone, and/or diaphragms determine in addition the spatial sensitivity of the photoreceptor. The spectral sensitivity, another important characteristic of photoreceptors, is mainly determined by the absorption spectrum of the visual pigment, but spectral filters and other optical factors can have distinctly modulatory effects. Although the basic principles of photoreception appear to be universal for animal vision, an enormous diversity of eyes exists, especially in the invertebrate kingdom. This indicates that the constraints for realising a functional visual organ are not very strict.

Invertebrates encompass prokaryotes, single-celled organisms, as well as highly developed eukaryotes, organisms with a complex neural organisation. Accordingly, photodetection can be quite primitive, based on membrane patches with light-sensing rhodopsin molecules, as in *Chlamydomonas* (Nagel *et al*., 2003), or involve accumulation and processing of optical information mediated by an advanced visual system (Land and Nilsson, 2002). In the latter case we can distinguish between the single-lens eyes of, for example octopus, where the lens maps the environment onto a vast array of photoreceptors, and the compound eyes of crustaceans and insects, equipped with numerous facet lenses that focus light on a limited number of photoreceptors, usually eight or nine. The set of photoreceptors in the latter case forms, together with the dioptric system and supporting cells, an eyelet, called an ommatidium. Almost any imaginable intermediate between the 'simple' single-lens and 'complex' compound eye type can be found in the invertebrate kingdom. For instance, spiders have eight single-lens eyes, together covering the optical environment, whereas the eyes of strepsipteran insects are composed of several lens–retinae assemblies that are neither well-focused systems nor compound eyes (Pix *et al*., 2000), although their origin may well be closely related (Buschbeck *et al*., 2003). Furthermore, in addition to their pair of compound eyes, insects often have three ocelli, small eyes with one ill-focused lens and a retinal array of photoreceptors (Land and Nilsson, 2002).

In the following sections, we will highlight the optical principles applied in invertebrate visual systems by considering a few exquisite, exemplary cases. The well-studied eyes of flies (specifically *Drosophila*) and butterflies (notably *Pieris rapae*) will serve as a scaffold for a more general discussion of invertebrate eyes. However, first a brief survey of invertebrate visual pigments will be given, because light absorption by the visual pigments is the central and fundamental process of vision and, furthermore, the visual pigment absorption spectra play a crucial role in the tuning of other, accessory optical components of the eye.

## 1.2 INVERTEBRATE VISUAL PIGMENTS

### 1.2.1 Visual pigment chromophores

Visual pigments are chromoproteins with a molecular weight of about 40 kD, due to *c*. 380 amino acids, one of which covalently binds the 11-cis isomer of a light-sensitive prosthetic group referred to as the chromophore. The main chromophore of both invertebrate and vertebrate visual pigments is retinal (RAL1), the aldehyde of vitamin A1 (retinol). 3,4-didehydroretinal (RAL2), commonly encountered in vertebrates (fish, amphibia, and reptiles), has been identified in crayfish as well (Suzuki and Makino-Tasaka, 1984). Many insect species, for example flies and butterflies, employ instead 3-hydroxyretinal (RAL3), derived from 3-hydroxyretinol, vitamin A3 (Vogt, 1989). This even occurs in two enantiomeric forms, (3R)- and (3S)-11-cis 3-hydroxyretinal (Seki and Vogt, 1998). Both RAL1 and RAL3 have been found in dragonflies (Seki *et al*., 1989) and fireflies (Hariyama *et al*., 1998). In a cephalopod, the firefly squid *Watasenia scintillans*, (4R)-4-hydroxyretinal (RAL4) exists in addition to RAL1 and RAL2 (Matsui *et al*., 1988; Seidou *et al*., 1990; reviews: Gärtner, 2000; Yokoyama and Yokoyama, 2000). The visual pigments have received various names, depending on both the absorption spectrum and their chromophore, but it is a matter of convenience to use simply rhodopsin as the generic name for all visual pigments.

Light-sensing rhodopsin-like pigments exist in lower organisms, for example in the unicell *Chlamydomonas*, where two rhodopsins using RAL1 act as light-switched ion channels and mediate phototaxis and photophobic responses (Nagel *et al*., 2003). The parent state exists in the all-trans form, which is photoconverted to the 13-cis configuration (Gärtner, 2000).

### 1.2.2 Visual pigment absorption spectra

Whereas all five types of natural chromophores by themselves absorb exclusively in the ultraviolet, the absorption spectrum of a visual pigment can extend anywhere from the ultraviolet (UV) to well into the red. The visual pigments absorbing appreciably at wavelengths visible for the human eye have an absorption spectrum consisting of two bands, a main α-band, half-width about 100 nm, and a secondary β-band, which is restricted to the UV and has an amplitude much smaller than that of the α-band.

Extensive comparative research on the absorption spectra of several different visual pigments has shown that the spectral shape can be well described by rather simple mathematical formulae, called templates, containing only one parameter, the peak wavelength of the α-band, $\lambda_{max}$ (e.g. Stavenga *et al*., 1993; Govardovskii *et al*., 2000). The fortuitous property of visual pigments that a single value characterises the spectral shape must be intrinsic to the molecular structure and amino acid composition, but in which way is unknown. Figure 1.1A shows the absorption spectra of visual pigments absorbing maximally in the UV, blue (B), and green (G) wavelength range, with peak wavelengths $\lambda_{max} = 350$, 440, and 530 nm, respectively. Accordingly the rhodopsins are called R350, R440, and R530. The main α-band and the smaller β-band are clearly seen in the R530 spectrum. For R440, the β-band merges with the α-band, whilst the two bands are no longer distinguishable in the spectrum of R350.

### 1.2.3 Visual pigment photochemistry and phototransduction

A successful capture of a photon by a rhodopsin molecule causes the chromophore to isomerise from the 11-cis to the all-trans form. This is followed by thermally driven conformational changes of the opsin, which end in the metarhodopsin state, because invertebrate metarhodopsins are thermostable, at least for several minutes. In natural daylight conditions this longevity is sufficient for the visual pigment molecules to absorb another photon, which then results in reconversion of the metarhodopsin into the native rhodopsin state.

The photochemical conversions of the rhodopsins to their metarhodopsins and vice versa proceed via a number of intermediary states, within a time span of a few milliseconds. The molecular pathways seem to be very similar for rhodopsin to metarhodopsin conversion in both

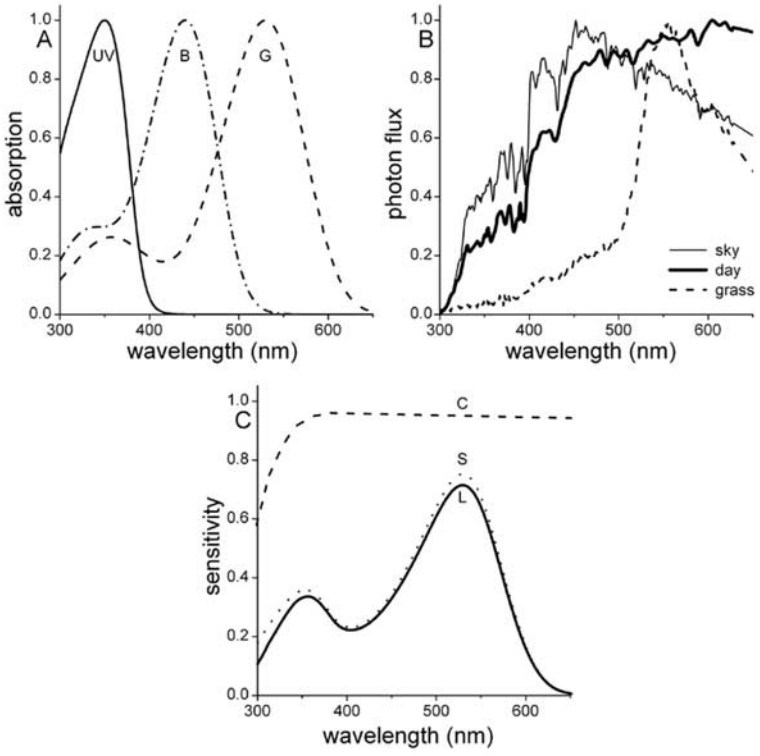


Fig. 1.1 Visual pigment absorption spectra, environmental radiance spectra, and the tuning of light sensitivity to environmental light. A. A set of typical visual pigment spectra, characteristic of hymenopteran insects (generated with the template of Govardovskii *et al.*, 2000). Bees and bumblebees generally employ visual pigments absorbing in the ultraviolet (UV), blue (B), and green (G) wavelength range (Peitsch *et al.*, 1992). Similar sets are found in many other invertebrate species, but species with different numbers of visual pigment types also occur in the invertebrate kingdom. B. A daylight spectrum (*bold* line) is the resultant of a variety of environmental radiance spectra (e.g. the blue sky and the green grass). C. The transmittance spectrum of a moth cornea (C) multiplied by the averaged spectrum (S) of a set of bee photoreceptors, consisting of 1 UV, 1 B, and 6 G receptors, yields a light sensitivity spectrum (L), which resembles a weighted average of environmental spectra with an emphasis on the green wavelength range.

vertebrates and invertebrates (Hamdorf, 1979; Stavenga *et al.*, 2000; Vought *et al.*, 2000). However, whereas invertebrate metarhodopsins are thermally stable, vertebrate metarhodopsins rapidly thermally decay, resulting in a complete separation of opsin and chromophore.

The peak absorption wavelengths of invertebrate rhodopsins range from the UV to the orange. An interesting, but not yet explained fact, is the intimate relation between the peak wavelengths of the rhodopsins and their metarhodopsins. When $\lambda_{max}(R) < 500$ nm, then $\lambda_{max}(M) > \lambda_{max}(R)$, that is, the metarhodopsins of UV and blue rhodopsins are bathochromic (long-wavelength) shifted; and if $\lambda_{max}(R) > 500$ nm then $\lambda_{max}(M) < \lambda_{max}(R)$, that is, the metarhodopsins of green and orange rhodopsins are hypsochromic (short-wavelength) shifted (Fig. 1.2).

The absorption amplitude of the $\alpha$-band of metarhodopsin is usually much higher than that of the native rhodopsin, due to the all-trans configuration of the chromophore (Hamdorf, 1979). The molar absorbance of invertebrate rhodopsins, $\varepsilon_{max}(R)$, varies from 34 000 to 43 000 $M^{-1}$ $cm^{-1}$ (Stavenga and Schwemer, 1984). The $\varepsilon_{max}(M)$ of insect

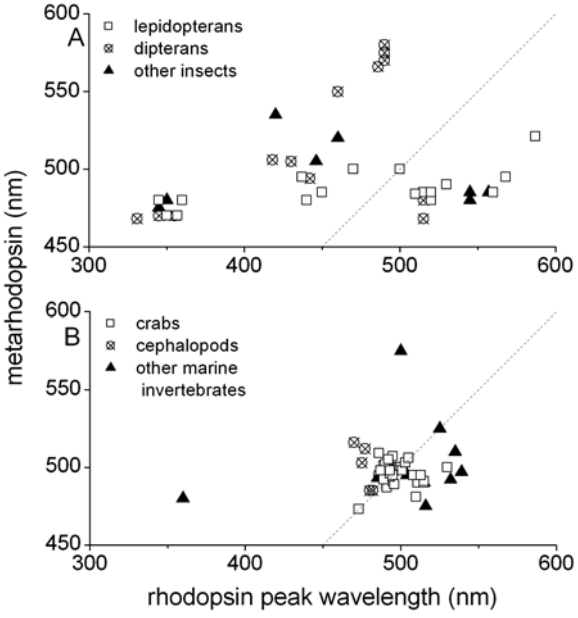


Fig. 1.2 Spectral properties of invertebrate visual pigments. A. Relationship between the peak wavelengths of the rhodopsins and their metarhodopsins in insects. Whereas $\lambda_{max}(R)$ ranges from the UV to the orange, the range of $\lambda_{max}(M)$ is distinctly narrower. When $\lambda_{max}(R) < 500$ nm then $\lambda_{max}(M) > \lambda_{max}(R)$, and when $\lambda_{max}(R) > 500$ nm then $\lambda_{max}(M) < \lambda_{max}(R)$. B. The same rule appears to hold for marine invertebrates, but the marine environment seems to condition the range of visual pigment peak wavelengths to a region around 500 nm.

metarhodopsins is higher by a factor of about 1.7, similar to the ratio between the molar absorbances of 11-cis and all-trans-retinal, being 24 900 and 43 400 $M^{-1}\,cm^{-1}$, respectively. However, $\varepsilon_{max}(M)/\varepsilon_{max}(R)$ of cephalopods and crayfish is distinctly lower, 1.2 to 1.5 (Stavenga and Schwemer, 1984), and in many crabs, this ratio is close to 1 (Cronin and Forward, 1988), possibly due to incomplete isomerisation.

Rhodopsin conversion and metarhodopsin reconversion, occurring in a visual pigment-containing tissue, can be directly monitored by measuring the changes in the absorption spectrum and then calculating absorbance difference spectra. Study of the photochemistry of visual pigments in vivo is possible via the distinct fluorescence of invertebrate metarhodopsins, which is also a convenient means to visualise the organelles that contain the visual pigments (Cronin and Goldsmith, 1982; Stavenga *et al*., 1984; Oberwinkler and Stavenga, 1998).

The metarhodopsin can bind a G-protein, and this step forms the start of the phototransduction process (Hardie, 2001; Chapter 2). The affinity for G-protein coupling is short-lived, however, due to inactivation of metarhodopsin by phosphorylation and binding of an arrestin molecule (Hardie, 2001; Huber, 2001). Photon absorption by the inactivated metarhodopsin results in an inactive rhodopsin state, but subsequent arrestin shedding and dephosphorylation yields a rhodopsin that is ready for another round in triggering the phototransduction chain. In case metarhodopsin is not photoreconverted, it is enzymatically degraded and subsequently replaced by newly synthesised rhodopsin. Optical measurements have demonstrated that completion of this pathway takes several minutes to hours, depending on the species (Bernard, 1983; Schwemer, 1989; Vanhoutte and Stavenga, 2005).

### 1.2.4 Sensitising pigments

A special photochemical pathway has been developed by higher Diptera. They have a class of visual pigment that is unique among both invertebrate and vertebrate rhodopsins, because it employs a sensitising pigment. The main blue-green rhodopsin of *Drosophila*, Rh1 (Table 1.1), binds 3-hydroxyretinol, which absorbs in the UV (Kirschfeld *et al*., 1977). It functions as a second chromophore, as it transfers the energy absorbed from a UV-photon to the principal chromophore, 3-hydroxyretinal. When the latter is in the 11-cis hydroxyretinal configuration, the transferred energy causes molecular excitation and

Table 1.1. *Visual pigments in* Drosophila

| Rhodopsin | Rh1 | Rh2 | Rh3 | Rh4 | Rh5 | Rh6 |
|---|---|---|---|---|---|---|
| Location | R1–6, R7r | ocelli | R7, 8 marg, R7p | R7y | R8p | R8y |
| R | 486 | 418 | 331 | 355 | 442 | 515 |
| M | 566 | 506 | 468 | 470 | 494 | 468 |

Rh1 is the main, blue-green-absorbing visual pigment of the fruitfly, located in the peripheral photoreceptors, R1–6, as well as in the central photoreceptor of the dorsal region, R7r (i.e. the central photoreceptor that looks red with fluorescence light microscopy). The violet-blue-absorbing Rh2 exists in the ocelli. The UV visual pigment Rh3 is found in the dorsal eye margin in both central photoreceptors (R7marg and R8marg). It also occurs elsewhere in the eye in R7p photoreceptors (i.e. those looking pale in transmission), but the other class, R7y (looking yellow in transmission), contains a slightly different UV visual pigment, Rh4; the corresponding R8 photoreceptors use blue (Rh5) and green (Rh6) rhodopsins, respectively. The third and fourth rows show $\lambda_{max}$, the wavelength (in nm) where the rhodopsin (R) and metarhodopsin (M) absorb maximally (Hardie, 1985; Salcedo *et al*., 1999).

chromophore isomerisation, leading to the usual rhodopsin–metarhodopsin conversion (and the subsequent triggering of the phototransduction chain). Similarly, energy transfer from the 3-hydroxyretinol to the all-trans chromophore of metarhodopsin results in reconversion of the metarhodopsin into rhodopsin (Minke and Kirschfeld, 1979).

The 3-hydroxyretinol, acting as a sensitising pigment for the visual pigment, strongly enhances the photoreceptor sensitivity in the ultraviolet (Hardie, 1985). However, because the UV content of natural illuminants is minor (Fig. 1.1B), the effective sensitivity enhancement is probably limited to about 10% (Stavenga, 2004b).

### 1.2.5 Visual pigments and the photic environment

Evidence is accumulating that insect visual pigments fall into three major clades, in line with the view that different molecular classes covering the three spectral ranges, UV, blue, and green–red, arose early in the evolution of insects (Briscoe and Chittka, 2001). Which visual pigments an animal adopts is presumably dependent on the spectral distribution of light in its environment, commonly characterised by a so-called daylight spectrum (Lythgoe, 1972). A daylight

spectrum (as that in Fig. 1.1B) gives the irradiance, the light flux arriving at a surface from all directions, with dimension photons per unit time and area. The irradiance is negligible below 300 nm, rather low in the ultraviolet, and steadily climbs with increasing wavelength. Although a daylight spectrum is frequently used in visual analyses, it does not necessarily represent the spectral light distribution to which a visual system is tuned, because photoreceptors receive radiance. A photoreceptor in the dorsal eye area of a flying bee normally detects the radiance of the blue sky, whilst a ventral photoreceptor samples the green grass (Fig. 1.1B). The composition of the spectral receptors of a visual system may hence depend on the eye region and the piece of environment in the habitat that the photoreceptors observe on average.

The three rhodopsins of Fig. 1.1A are exemplary for the case of the honey bee, *Apis mellifera* (Peitsch *et al*., 1992). The honey bee has eight main photoreceptor types, where the majority have a green-absorbing rhodopsin (G, Fig. 1.1A), whilst an UV- and a blue-absorbing (B) rhodopsin serve a small minority of the photoreceptors. With a ratio UV:B:G = 1:1:6 (Section 1.5), the weighted sum of the three absorption spectra yields spectrum S of Fig. 1.1C, assuming that the peak absorption coefficient of the three rhodopsins is identical. Figure 1.1C shows that the bee photoreceptors together cover a broad wavelength range, from the UV to the orange-red, with a distinct preference for the green. Considering that an important task of bee eyes is to discriminate flowers in a green world, this may suggest that the dominance of green receptors in the bee eye is an adjustment to the weighted sum of the photon fluxes from different natural sources. In fact, a weighted sum of the environmental radiance spectra of Fig. 1.1B with a dominant green component approximates spectrum S of Fig. 1.1C.

A point to consider here is that various optical factors will modify the effective light flux reaching the photoreceptors. The first one is the dioptric system, which in the bee consists of a facet lens and a crystalline cone. These elements not only act together as a light-focusing system, but also as a spectral filter, because they are not fully transparent. As an example, Fig. 1.1C shows the transmittance of the cornea (C), of a moth (Bernhard *et al*., 1965), which is high throughout most of the visible wavelength range, but rapidly decreases at shorter wavelengths. Multiplication of sensitivity spectrum S with corneal transmittance C yields sensitivity spectrum L, which is somewhat reduced, especially near 300 nm. This reduction will be unimportant for vision, however, because the photon flux in nature is minor in the

far-UV (Fig. 1.1B). Low transmittance of the dioptric system in the far-UV will therefore not seriously limit sensitivity.

A set of three visual pigments seems in general to be sufficient for covering the main visual wavelength range, as this situation is not only encountered in the bee, but in many invertebrate species, for example the moth *Manduca sexta* (White *et al*., 2003) and the butterfly *Vanessa cardui* (Briscoe *et al*., 2003). However, the spectral sensitivities of the photoreceptors have often been diversified, by adding visual pigment types (Kitamoto *et al*., 2000; Briscoe, 2001) and/or by applying spectral filters (e.g. Marshall *et al*., 1991; Arikawa *et al*., 1999a, 2005; review: Briscoe and Chittka, 2001).

The set of three rhodopsins of Fig. 1.1B is adequate for colour vision, but the wavelengths of maximal colour discrimination depend on the relative spectral positions of the different rhodopsins. The fine tuning of the absorption peaks presumably occurs by tinkering with special amino acids, located near the retinal chromophore, as the intramolecular interactions are responsible for the visual pigments' spectral characteristics (Briscoe, 2001). Tuning of its visual pigment absorption spectra to the animal's needs will be an important and ongoing aspect in the evolution of vision.

## 1.3 INVERTEBRATE SCREENING PIGMENTS

### 1.3.1 Pigment classification and location

When the only pigments in an eye are visual pigments, it will perform rather poorly. Virtually any eye therefore contains high concentrations of darkly coloured, photostable pigments, the so-called screening pigments. Their function is to prevent the absorption of stray light by the visual pigments, because that would degrade spatial acuity. The screening pigments of insect eyes include the (quite appropriately named) ommochromes, pterins, and carotenoids (Kayser, 1985). Crustaceans derive their ocular pigments from the same classes, and also utilise melanins and purines (Shaw and Stowe, 1982). The pigments are bound to proteins concentrated in granules, cross-section *c.* 0.5 μm, which densely pack the screening pigment cells. The primary pigment cells of insect eyes envelop the crystalline cone, whilst the secondary pigment cells stretch from cornea to basement membrane, the limiting layer proximal to the retina. In crustacean eyes, the corresponding pigment layers are sometimes called distal and proximal pigment cells (see Stavenga, 1989).