Rhabdom evolution in butterflies: insights from the uniquely tiered and heterogeneous ommatidia of the Glacial Apollo butterfly, *Parnassius glacialis*

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The eye of the Glacial Apollo butterfly, *Parnassius glacialis*, a ‘living fossil’ species of the family Papilionidae, contains three types of spectrally heterogeneous ommatidia. Electron microscopy reveals that the Apollo rhabdom is tiered. The distal tier is composed exclusively of photoreceptors expressing opsins of ultraviolet or blue-absorbing visual pigments, and the proximal tier consists of photoreceptors expressing opsins of green or red-absorbing visual pigments. This organization is unique because the distal tier of other known butterflies contains two green-sensitive photoreceptors, which probably function in improving spatial and/or motion vision. Interspecific comparison suggests that the Apollo rhabdom retains an ancestral tiered pattern with some modification to enhance its colour vision towards the long-wavelength region of the spectrum.

**Keywords:** vision; colour; motion; compound eye; photoreceptor; opsin

1. INTRODUCTION

Insect compound eyes are composed of units called ommatidia. In butterflies, an ommatidium bears nine photoreceptors R1–9, constructing a fused and tiered rhabdom. Typically, the distal and proximal tiers of the rhabdom are made up of the microvilli of R1–4 and R5–8 photoreceptors, respectively. R9 is a small basal photoreceptor adding a few microvilli at the base of the rhabdom [1–3]. The ommatidia are very similar in cellular arrangement, but they are in fact spectrally heterogeneous [4–7]. Since the first detailed report of the spectral heterogeneity of butterfly ommatidia in the Japanese yellow swallowtail, *Papilio xuthus* [8,9], similar heterogeneity has been demonstrated in four butterfly families: Papilionidae (*Papilio glaucus* [10]), Pieridae (*Pieris rapae* [11]), Anthocaris *scolymus* [12], *Colias erate* [13]), Nymphalidae (*Vanessa cardui* [14], *Danaus plexippus* [15], *Heliconius erato* [16]) and Lycaenidae (*Lycaena rubidus* [17]). The accumulated evidence indicates that the eyes of most butterflies are composed of three randomly distributed types of ommatidia.

While the ommatidial heterogeneity itself appears to be a common feature, details of the physiological and molecular organization are quite diverse among species. The diversity includes the number of opsin genes, their expression pattern, the eye pigmentation and spectral sensitivities of the photoreceptors. In order to elucidate the origin of the various butterfly eye designs, we have initiated a study on the Glacial Apollo butterfly, *Parnassius glacialis*, a ‘living fossil’ species in the family Papilionidae [18]. The Apollo belongs to the Parnassini, the oldest tribe in the subfamily Parnassiinae, which diverged from the other papilionid subfamilies in the Cretaceous period [19] and rapidly radiated in the late Tertiary period [20].

In a previous study [18], we found that the eye of *Parnassius*, like all other butterfly eyes investigated so far, has three types of ommatidia (table 1). It expresses four mRNAs encoding visual pigment opsins, one ultraviolet- (UV) (PgUV), one blue- (PgB) and two long (PgL2, L3) wavelength-absorbing types. The R3–4 photoreceptors of type I and II ommatidia express PgL2, a green-absorbing visual pigment. PgL3, a presumptive red-absorbing visual pigment, exists in R3–4 photoreceptors of type III ommatidia whose rhabdom is surrounded by a red screening pigment [18]. This expression pattern is quite unexpected because it indicates that R3 and R4 are spectrally heterogeneous, whereas these photoreceptors in all studied butterflies as well as in other insects have been found to express the same visual pigment. To address the question of how such an organization functions in vision, detailed anatomical information of the visual system is indispensable. We therefore carried out an anatomical study of the compound eye retina of *P. glacialis*.

2. MATERIAL AND METHODS

(a) Animals

Males of the Glacial Apollo, *P. glacialis* (Butler) were captured around Lake Tsukui, Kanagawa, Japan. We also used, for comparison, the common bluebottle, *Graphium sarpedon* (Linnaeus) and the Japanese yellow swallowtail, *P. xuthus* (Linnaeus), which were captured around the Sokendai-Hayama campus, Kanagawa, Japan.

(b) Histology

For light and electron microscopy, isolated eyes were pre-fixed in 2 per cent paraformaldehyde and 2 per cent glutaraldehyde...
Table 1. A summary of the characteristics of ommatidal types in *P. glacialis*. (n.a., data not available.)

<table>
<thead>
<tr>
<th>photoreceptor/tier</th>
<th>opsin/direction of microvilli</th>
<th>type Ia</th>
<th>type II</th>
<th>type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1/distal</td>
<td>PgB mostly straight</td>
<td>PgUV + PgB/curve, 90°</td>
<td>PgUV + PgB/curve, 90°</td>
<td></td>
</tr>
<tr>
<td>R2/distal</td>
<td>PgUV, curve, ± 30°</td>
<td>PgUV + PgB/curve, 90°</td>
<td>PgUV + PgB/curve, 90°</td>
<td></td>
</tr>
<tr>
<td>R3–4/proximal</td>
<td>PgL2/straight, 90°</td>
<td>PgL2/straight, 90°</td>
<td>PgL3/straight, 90°</td>
<td></td>
</tr>
<tr>
<td>R5–8/proximal</td>
<td>PgL2/straight, 45° or 135°</td>
<td>PgL2/straight, 45° or 135°</td>
<td>PgL3/straight, 45° or 135°</td>
<td></td>
</tr>
<tr>
<td>R9/basal</td>
<td>n.a./variable</td>
<td>n.a./variable</td>
<td>n.a./variable</td>
<td></td>
</tr>
<tr>
<td>rhabdom shape</td>
<td>bean</td>
<td>dumbbell</td>
<td>dumbbell</td>
<td></td>
</tr>
<tr>
<td>fluorescence</td>
<td>yes/particles</td>
<td>no</td>
<td>no</td>
<td></td>
</tr>
<tr>
<td>pigment</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>fraction (%)b</td>
<td>45</td>
<td>23</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

In type Ib, R1 and R2 are exchanged.

See Awata et al. [18].

in 0.1 mol l\(^{-1}\) sodium cacodylate buffer (CB; pH 7.3) for 120 min at 20–25°C. After a brief wash with CB, the eyes were postfixed in 2 per cent osmium tetroxide in CB for 2 h at 20–25°C. Following dehydration with acetone and infiltration with propylene oxide, eyes were embedded in Quetol 812 (Nissin EM, Tokyo) or Spurr’s resin (Polysciences, War-ington PA). For light microscopy, 5 μm-thick sections were observed with a light microscope (BX51, Olympus, Tokyo) equipped with a DP71 camera system (Olympus). Ultrathin sections double-stained with uranyl acetate and lead citrate were observed in a transmission electron microscope (H7650, Hitachi, Tokyo). In a series of electron micrographs obtained from a single eye, we quantified the cross-section of the rhabdom and its composing rhabdomeres at each depth using iTEM software (Soft Imaging System, Riverside, CA, USA).

**Fluorescence microscopy**

We observed the ommatidial fluorescence in the intact eye of butterflies mounted on the stage of a fluorescence microscope (BX60, Olympus). The microscope was equipped with a dichroic cube U-MWU (band-pass filter at 350 and cut-off filter at 420 nm) and a DP21 camera (Olympus). To identify the fluorescing ommatidia, we put silvery ink around the illuminated region, which facilitated further anatomical processing for light and electron microscopy as described already.

To observe the ommatidial fluorescence in sections of the eyes of *P. xuthus*, isolated eyes were pre-treated in 2 per cent paraformaldehyde and 2 per cent glutaraldehyde in 0.1 mol l\(^{-1}\) sodium phosphate buffer (PB; pH 7.4) for 30 s in total (6 × 5 s) with a microwave oven to enhance fixative penetration. The eyes were further fixed for 20 min in the same fixative at 4°C. After being infiltrated in 30 per cent sucrose in PB for cryoprotection, the eyes were frozen in OCT compound (Sakura Finetek Japan, Tokyo), and cut with a cryostat (HM500 OM, Micro-edge Instruments, Tokyo).

3. RESULTS

A compound eye of *P. glacialis* consists of about 5000 ommatidia, whose length varies between 200 and 500 μm, depending on the eye region. We investigated the ommatidia in the fronto-ventral region, where the thickness of the photoreceptor layer is about 400 μm and that of the dioptric apparatus (cornea and crystalline cone) is about 80 μm (figure 1a). The ommatidium contains nine photoreceptors, R1–9. The cell bodies of R1–8 stretch over the entire length of the ommatidium, while R9 appears only at the region immediately distal to the basement membrane. The rhabdomeral microvilli of all photoreceptors together form the fused and tiered rhabdom.

(a) **Heterogeneity of ventral ommatidia**

Figure 1b shows a transverse section of the retina at a depth of about 180 μm from the corneal surface, that is, at about 100 μm from the rhabdom tip. The profiles of R1 and R2 cell bodies are evident at this level, whereas the cell bodies of the R3–8 are small and hardly distinguishable. As we described previously [18], the ommatidia can be divided into three types; I, II and III, based on two features: the size of R1 and R2 cell bodies and the perirhabdomal pigmentation (table 1). First, in type I ommatidia, the cross sections of R1 and R2 substantially differ. Either R1 is smaller than R2 (ommatidium Ib in figure 1b) or *vice versa* (ommatidium Ib in figure 1b). The ratio of the numbers of Ia and Ib ommatidia is about 1 : 1. The cell bodies of R1 and R2 cell are similar in size in ommatidial types II and III. Only type III has characteristic pigmentation around the rhabdom. The pigment colour is reddish; the colour is not obvious in figure 1b,c because these are sections from an osmicated tissue.

Figure 1c is a transverse section of the same eye region as that of figure 1b, but at a deeper level of about 300 μm from the corneal surface. The cell bodies of R3–8 are enlarged here, and the (reddish) pigment in type III ommatidia clearly extends to this level (arrowheads).

(b) **Ultrastructure of the rhabdom**

We analysed ultrathin transverse sections of ommatidia at every 20 μm along the entire length of the rhabdom. Figure 2 shows three representative sets of serial sections, each from a particular type of ommatidium. Actual depths at which each picture was taken are indicated at the bottom left-hand corner of the pictures. All three ommatidial types can be easily discriminated at any depth shown here. Figure 2a–d shows four consecutive images of the rhabdom of a type Ia ommatidium (figure 1b). As is clearly seen from the distance between belt desmosomes at the rhabdomere base, the rhabdomere of R2 is larger than that of R1 in the distal region (figure 2b, arrowheads). The microvilli of R1 are mostly straight, while the microvilli of R2 are curved and split into two halves, which fan out in the direction of +30° and −30° relative to the vertical axis of the eye. At a depth...
of about 330 μm, the R1 rhabdomere becomes larger, and R3–4 photoreceptors start to extend microvilli to form rhabdomeres, while the R5–8 do not yet have microvilli (figure 2c). At a depth of about 400 μm, the R5–8 contribute diagonally oriented microvilli to the rhabdom. The R2 rhabdomere disappears at this level (figure 2d).

Contrary to type I ommatidia, both type II (figure 2e–h) and type III (figure 2i–l) have R1 and R2 rhabdomeres of similar size. The most conspicuous difference between these two types is the red pigmentation as seen in light micrographs (figure 1b,c). The R3–8 photoreceptors of type III ommatidia contain pigment granules of irregular shape close to their rhabdomeres (figure 2i–k). Moreover, the rhabdom of type III is distally, immediately below the crystalline cone, much larger than the rhabdoms in types I and II (figure 2i). At 200–300 μm, the rhabdom exhibits a characteristic dumbbell shape in both types II and III.

The distal rhabdom of types II and III is composed exclusively of the microvilli of R1 and R2, which fan out almost horizontally (figure 2f,g,i,k). R3 and R4 start to contribute their microvilli to the rhabdom at around 300 μm, and at 400 μm the rhabdom is made up of exclusively R3–8 microvilli in both types (figure 2h,i).

To quantify the organization of tiers and to assess the relative contribution to light sensitivity of photoreceptors, we measured the cross-sectional area of rhabdoms and estimated the fraction of each rhabdomere at various depths in electron micrographs. The size of the rhabdomeres was then calculated by multiplying the fraction of each by the total rhabdom area at each depth (figure 3). In type I, the rhabdom area increases from 1.5 μm² at 100 μm depth to 4.5 μm² at 250 μm. It slightly decreases at around 300 μm, and it again increases towards the basal region (figure 3a). The rhabdom area of type II peaks at 200–250 μm, decreases in the middle layer down to about 3.5 μm² and increases again towards the basal region (figure 3b). The rhabdom area of type III peaks at 150–250 μm, then decreases in the middle layer to 2.5 μm² and subsequently increases again (figure 3c). The rhabdoms of type II and III have a clear constriction around the border between the distal and proximal tier of the retina. The constriction is more pronounced in type III, which contains the red perirhabdomal pigment.

(c) Ultraviolet induced autofluorescence

A number of butterfly eyes exhibit fluorescence depending on the ommatidal type [11,21]. We therefore investigated whether the *Parnassius* eye also exhibited fluorescence. We found a subset of ommatidia emitting strong fluorescence under UV epi-illumination (figure 4a). Figure 4b shows a transverse semi-thin section of the region shown in figure 4a, demonstrating that the fluorescing ommatidium is of type Ia (figure 4a,b, see also figure 1b). To uncover the physical basis of the fluorescence, we carefully inspected electron micrographs of rhabdoms. It thus appeared that the rhabdom of type I ommatidia often contain particles of 30–40 nm in diameter. The particles have no membrane, and exist mostly in the distal 100 μm of the rhabdom, in the extracellular space within or around the rhabdom (figure 4c).

The UV-induced fluorescence was first discovered in *Papilio*, in their type II ommatidia [9,21], but the origin of the fluorescence has not been identified so far. Because the anatomy of *Parnassius* suggested that the fluorescence is localized in characteristic particles, we decided to re-examine the electron micrographs of *Papilio* eyes. We thus found particles accumulated in type II ommatidia as four clusters in the extracellular space in the most distal region of the rhabdom (figure 4d,e). A frozen section observed with a fluorescence microscope applying UV-excitation revealed four fluorescing spots, corresponding to the particle clusters (figure 4f) [22]. We also examined the eye of another papilionoid species, *G. sarpedon*, and found fluorescing ommatidia, which contain particles similar to those of the eyes of *Parnassius* and *Papilio* in the extracellular space (arrowheads in figure 4g).

4. DISCUSSION

(a) R3 and R4 are proximal, long-wavelength receptors

The most curious feature of *Parnassius* ommatidia is the organization of the tiered rhabdom. In all butterfly
species studied so far, the distal tier of the rhabdom is formed by the microvilli extending from four photoreceptors, R1–4, while the R5–8 photoreceptors are usually contributing only to the proximal rhabdom [3,12,23–25]. In a few nymphalid species, R3 and R4 contribute microvilli to both the distal and proximal rhabdom [14,26–28]. However in *Parnassius*, the distal rhabdom is composed exclusively of the microvilli of R1 and R2 photoreceptors in all three ommatidial types, and R3 and R4 photoreceptors contribute their microvilli exclusively to the proximal rhabdom.

Figure 2. Electron micrographs of serial sections of type I (*a–d*), II (*e–h*) and III (*i–l*) ommatidia at ca 120 (*a,e,i*), 200 (*b,f,j*), 300 (*c,g,k*) and 400 μm (*d,h,l*) depth. Rh indicates the rhabdom. Scale bar, 1 μm.
in the proximal tier, together with R3–8 photoreceptors (figures 2 and 3). In *Parnassius*, R3 and R4 are therefore proximal photoreceptors.

The organization of the rhabdom tiers is probably related to the localization of long-wavelength-absorbing visual pigments. In addition to a UV- and a blue-absorbing visual pigment, the eyes of *Parnassius* express two long-wavelength-absorbing visual pigments, PgL2 and PgL3, orthologues of *Papilio* PxL2 (515 nm-absorbing) and PxL3 (575 nm-absorbing), respectively [18]. *In situ* hybridization revealed that the PgL2 (presumptive green) mRNA is expressed in R3–4 and R5–8 of type I and II ommatidia, while the PgL3 (presumptive red) mRNA exists in R3–4 and R5–8 of the red-pigmented type III ommatidia [18]. In *P. xuthus*, the PxL3 is expressed in R5–8 proximal receptors of type I ommatidia. The spectral sensitivity of the PxL3-expressing receptors, which peaks at 600 nm, has a narrower profile than the absorption spectrum of a visual pigment peaking at 600 nm. This difference was explained by assuming that the perirhabdomal red pigment acts as a red filter in front of a 575 nm-peaking visual pigment [23]. The perirhabdomal pigment of *P. xuthus* effectively acts as a filter only for the proximal photoreceptors, as was also found in the Small White butterfly, *P. rapae*. The distal R3 and R4 as well as the proximal R5–8 photoreceptors in all ommatidia of *Pieris* express a 560 nm-peaking visual pigment, PrL [29]. Whereas the spectral sensitivity of the distal R3–4 well matches the predicted absorption spectrum of a 563 nm visual pigment, the peak sensitivity of the proximal photoreceptors is shifted to 620 nm in the orange-pigmented ommatidia, and to 640 nm in the red-pigmented ommatidia [30]. This indicates that the effect of the perirhabdomal pigment is negligible in the distal tier, but the filtering effect is prominent in the proximal tier. In our previous study on the opsin expression in *Parnassius*, it was therefore rather puzzling to find that the PgL3 mRNA was expressed in R3 and R4 in the red-pigmented type III ommatidia of *Parnassius* [18]. These cells were expected to be distal photoreceptors, but the present anatomy has revealed that R3 and R4 are in fact proximal receptors. Accordingly, we conclude that all R3–8 photoreceptors in the type III ommatidia are most likely red receptors. Interestingly, the rhabdom of type III ommatidia has a strong constriction between the distal and the proximal tiers (figure 3c). The constriction most probably enhances the filtering effect of the pigment on the proximal receptors. On the other hand, the PgL2-expressing R3–8 photoreceptors in type I and II ommatidia are predicted to be green sensitive.

(b) The distal photoreceptors, R1 and R2

The distal R1 and R2 photoreceptors are short wavelength-sensitive. In type I ommatidia, R1 and R2 form sub-tiers within the distal tier (figure 3a): in the most distal region, the rhabdom is dominated by the rhabdomere of the PgUV-expressing R1 (in type Ib, or R2 in type Ia), while the region deeper than 300 μm is dominated by the PgB-expressing R2 (or R1 in type Ia), which has microvilli down into the proximal tier. The microvilli of the PgB-expressing cells are straight and parallel to the animals’ dorso-ventral axis at least in the distal and the proximal part of the ommatidia (figure 2a,d), suggesting the cell retains polarization sensitivity to some extent.

The type I ommatidia bear small non-membranous particles around the rhabdom (figure 4c), which fluoresce under UV epi-illumination (figure 4a), as in other papilionid species (figure 4e,f,g). The particles most likely contain 3-hydroxyretinol, the fluorescing material in the eye of *Papilio* [21]. We could not find similar particles in the subset of ommatidia of male *Pieris* that fluoresces under 420 nm epi-illumination [11].

The 3-hydroxyretinol in *Papilio* eyes acts as a far-UV-filter for the photoreceptors, which express a UV-absorbing visual pigment and thus become 400 nm-peaking violet receptors [21]. Therefore, the PgUV...

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**Figure 3.** Entire rhabdom areas (thick line) and rhabdomere areas of R1–8 in: (a) type I (open circles denote total Rh area; filled triangles, R1 (large at distal); inverted triangles, R2 (small at distal); open triangles, R3 + 4; filled circles, total R5–8); (b) type II (open circles denote total Rh area; filled triangles, R1 + 2; open triangles, R3 + 4; filled circles, total R5–8); and (c) type III (open circles denote total Rh area; filled triangles, R1 + 2; open triangles, R3 + 4; filled circles, total R5–8) ommatidia. The rhabdomere areas were calculated by multiplying rhabdom areas by rhabdomere fractions, which were estimated from typical electron micrographs at each depth. Data are means ± s.e.
expressing photoreceptors in the *Parnassius* type I fluorescing ommatidia may be expected to have a narrower spectral sensitivity profile with peak wavelength shifted to a longer wavelength than that of the so-called UV receptors. In fact, we have encountered in our preliminary electrophysiological recordings photoreceptors with 380 nm-peaking, narrow sensitivity spectra and with maximal polarization sensitivity parallel to the animals’ dorso-ventral axis.

In *Papilio*, coexpression of PxL2 (green) and PxL3 (red) visual pigment causes broad-band receptors [31], and therefore the R1 and R2 photoreceptors of *Parnassius* in type II and III ommatidia in the ventral region, which coexpress PgUV and PgB [18], may similarly be expected to have a broad sensitivity band, in the short wavelength region. We have detected some photoreceptors whose spectral sensitivity peaks at 360 nm with a broad sideband in the blue-green (450–550 nm) wavelength region with a reduced polarization sensitivity. These cells are yet to be localized by dye injection, but they are most likely the R1 and R2 of type II and III ommatidia whose microvilli are strongly curved (figure 2f, g, j, k).

(c) **Evolution of rhabdom tiers**

The present study on *Parnassius* provides some important implications about the evolution of tiered rhabdons. Figure 5 illustrates several forms of tiered rhabdons of butterflies, where the R1–9 photoreceptors contribute in different ways to the rhabdom, together with the rhabdom organization of flies, which have eight photoreceptors (figure 5a). The photoreceptor numbering system is different in butterflies and flies, but the numbering can be correlated based on the photoreceptor structures and differentiation processes [33]. Briefly, R1–6 in flies and R3–8 in butterflies have short axons terminating in the first optic ganglion, the lamina, and therefore are called short visual fibres (SVFs; short arrows in figure 5). The R1 and R2 of butterflies correspond to the fly R7, while the butterfly R9 corresponds to the fly R8. These photoreceptors are called long visual fibres (LVFs) because they extend their axons to the second optic ganglion, the medulla (long arrows in figure 5). The butterfly R3/4 is the ‘first photoreceptor pair’, which corresponds to the R2/5 pair in flies, which differentiates early in the developmental process, and the
The ancestral tier of the butterfly rhabdom is the counterpart of the fly R1–6 rhabdom. In (a) Drosophila and in (b–g) butterflies, the butterfly R1/2 pair and R9 correspond to the fly R7 and R8, respectively, with long axons terminating in the medulla (long arrows). Similarly, butterfly R3/4 pair and R5–8 set correspond to the fly R2/5 pair and the set of R1,3,4 and 6, all with short axons terminating in the lamina (short arrows). Each compartment indicates either R1′/2′ (fly-R7), R3/4′ (fly- R2′/5′), R5–8′ (fly-R1′/3′/4′/6′) or R9′ (fly-R8′), whose colour indicates opsin(s) it expresses: grey colours are UV opsins. R3/4 and R5–8 are merged when the opsins and locations in tiers are identical. Different colours in a single compartment indicate that the photoreceptors in the same category express different opsins in different ommatidia. (a) Drosophila rhabdomeres. Unlike butterflies, the rhabdomeres are not fused in flies. R1–6 are divided into two groups, the R2/5 pair and R1/3/4/6 quartet according to their differentiation process. The rhabdomeres of R1–6 form a trapezoid, whose centre is occupied by the rhabdomeres of R7 and R8 where the R7 rhabdomere sits on the top of the R8 rhabdomere. (b) Ancestral type of ommatidium with a bundle of short wavelength (UV and/or blue) sensitive R1/2 and green sensitive R3–8, not tiered except for the basal R9 photoreceptor. (c) Ancestral tiered rhabdom with short wavelength receptors located distally. (d) Rhabdom with modified proximal tier, as in Parnassius, with two opsins (green and red). The red perirhabdomal pigment is found in the ommatidia where R3–8 express the red opsins. See Awata et al. [18] for details. (e) Tiered rhabdom with an independent R3/4 green receptor system as in the painted lady, Vanessa cardui (Nymphalidae). (f) Rhabdom of the Japanese yellow swallowtail, P. xuthus (Papilionidae). Distally, it has specialized R3/4, expressing two duplicated green opsins. The proximal R5–8 express either one of green or red opsins, or coexpress both, which makes three spectrally heterogeneous ommatidia together with the red or yellow perirhabdomal pigments. See Arikawa [8] for details. (g) Rhabdom of the Small White, Pieris rapae (Pieridae). Three short wavelength opsins (UV, blue and violet) are expressed in R1/2 in the distal tier. Both the R3/4 pair and the R5–8 set express identical green opsins, but the spectral sensitivities of R5–8 are strongly modified by the red or orange perirhabdomal pigments. See Wakakuwa et al. [32] for details.
Specialization of these receptors is probably related to improved spatial and motion vision, visual modalities that are often based on an achromatic system that is ‘green’ sensitive [47–49]. The prominence of green sensitivity seems reasonable because the natural environment for flower-visiting insects is dominated by greenish light. For the best spatial resolution, the green receptors should exist in all ommatidia, thus completely filling the ommatidial lattice, and should also preferably occur in the distal tier where perihardamental pigments are incapable of modifying the spectral sensitivities. The R3/4 pair matches these criteria. In Vanessa, which has no perihardamental pigment, R3/4 bear rhabdomes along the entire length of the ommatidium (figure 5c).

The structure of the axons of R3/4 photoreceptors implicates their involvement in motion vision. In Papilio, the thick and smooth R3/4 axons make direct contacts with second-order visual interneurons in the lamina, which is probably an adaptation for fast signal processing. The four SVFs, R5–8, in contrast, have fine collaterals in the lamina with which they make mutual synaptic contacts, which indicates that at this level some degree of colour processing occurs [50,51]. Terminal specialization of the fast photoreceptor pair has also been found in honeybees [33,52], implicating its specialized function as well.

Taken together, the eye of P. glacialis is apparently unique because it lacks the monochromatic R3/4 system in the distal tier. Instead, the receptor set is enriched in terms of spectral sensitivities owing to L opsin duplication. Presumably for the slow-flying, ancestral group of butterflies, acute spatial and motion vision had a lower priority than extreme colour discrimination.

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