Review

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# The evolutionary diversity of insect retinal mosaics: common design principles and emerging molecular logic

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**Independent evolution has resulted in a vast diversity of eyes. Despite the lack of a common Bauplan or ancestral structure, similar developmental strategies are used. For instance, different classes of photoreceptor cells (PRs) are distributed stochastically and/or localized in different regions of the retina. Here, we focus on recent progress made towards understanding the molecular principles behind patterning retinal mosaics of insects, one of the most diverse groups of animals adapted to life on land, in the air, under water, or on the water surface. Morphological, physiological, and behavioral studies from many species provide detailed descriptions of the vast variation in retinal design and function. By integrating this knowledge with recent progress in the characterization of insect Rhodopsins as well as insight from the model organism *Drosophila melanogaster*, we seek to identify the molecular logic behind the adaptation of retinal mosaics to the habitat and way of life of an animal.**

## The homologous building blocks of insect retinas

Despite extensive differences in size, shape, and functional organization, all insect compound eyes share common ancestry and comprise the same repetitive structure, the unit eye or 'ommatidium' (see Glossary; reviewed in [1,2]). Ommatidia usually contain a fixed number of neuronal PRs as well as pigment cells (for optical isolation) and lens-secreting cone cells [2]. PRs contain light-sensitive Rhodopsins within specialized membrane compartments (rhabdomeres). In some species, rhabdomeres from each PR are optically isolated ('open rhabdom'), while in other species, rhabdomeres are fused together, forming a single light guide per ommatidium ('fused rhabdom') [3]. Variations in ommatidial design can also exist within the same retina, resulting in a retinal mosaic. The diverse types of retinal mosaics found in different species illustrate the complex interplay between the outcome of developmental patterning and the behavioral repertoire of the animal [4]. Three patterning strategies are common to most insect retinas and shape the retinal mosaic during development (Figure 1A): (i) in many cases, ommatidial subtypes with different chromatic sensitivities are distributed in a stochastic manner (reviewed in [5]); (ii) by contrast, close proximity to signaling factors emerging from the periphery of the retina often induces localized specialized ommatidia (reviewed in [6]); and (iii) finally, the insect retina can be divided into large territories, zones, bands, or stripes with different morphological or functional properties. Although great progress has been made in understanding the molecular genetic mechanisms governing both stochastic and localized specification in *Drosophila*, less is known about the formation of zones, bands, and stripe patterns observed in many species of insects. Here, we discuss how combining recent insight from *Drosophila* with descriptions of retinal morphology, visual behavior, and physiology from other insect species can lead to the identification of developmental principles.

A classification of insect PRs based on homology is difficult because of the scarcity of consistent traits that differentiate each subtype. Comparison of ommatidia from

## Glossary

**Dorsal rim area (DRA):** in many insect retinas, the DRA contains specialized photoreceptor cells mediating the behavioral response to linearly polarized skylight.
**Long visual fibers (Lvf):** photoreceptor cells whose axons project to the second neuropil in the insect brain, the medulla.
**Microvilli:** membranous invaginations of photoreceptor cells containing the visual pigments (Rhodopsins).
**Ommatidium:** the unit eye of insect compound eyes, containing neuronal photoreceptor cells, as well as pigment cells and cone cells.
**Rhabdom:** the light-guiding structure formed by individual photoreceptor membranes ('rhabdomeres') of an ommatidium. The rhabdom can be fused or 'open', if individual photoreceptors are optically isolated.
**Short visual fibers (Svf):** photoreceptor cells whose axons terminate in the first neuropil, the lamina.
**Ventral polarization area (VPA):** a specialized area in some insect retinas that contains photoreceptor cells mediating the behavioral response to linearly polarized light reflected from shiny surfaces, such as leaves or water.

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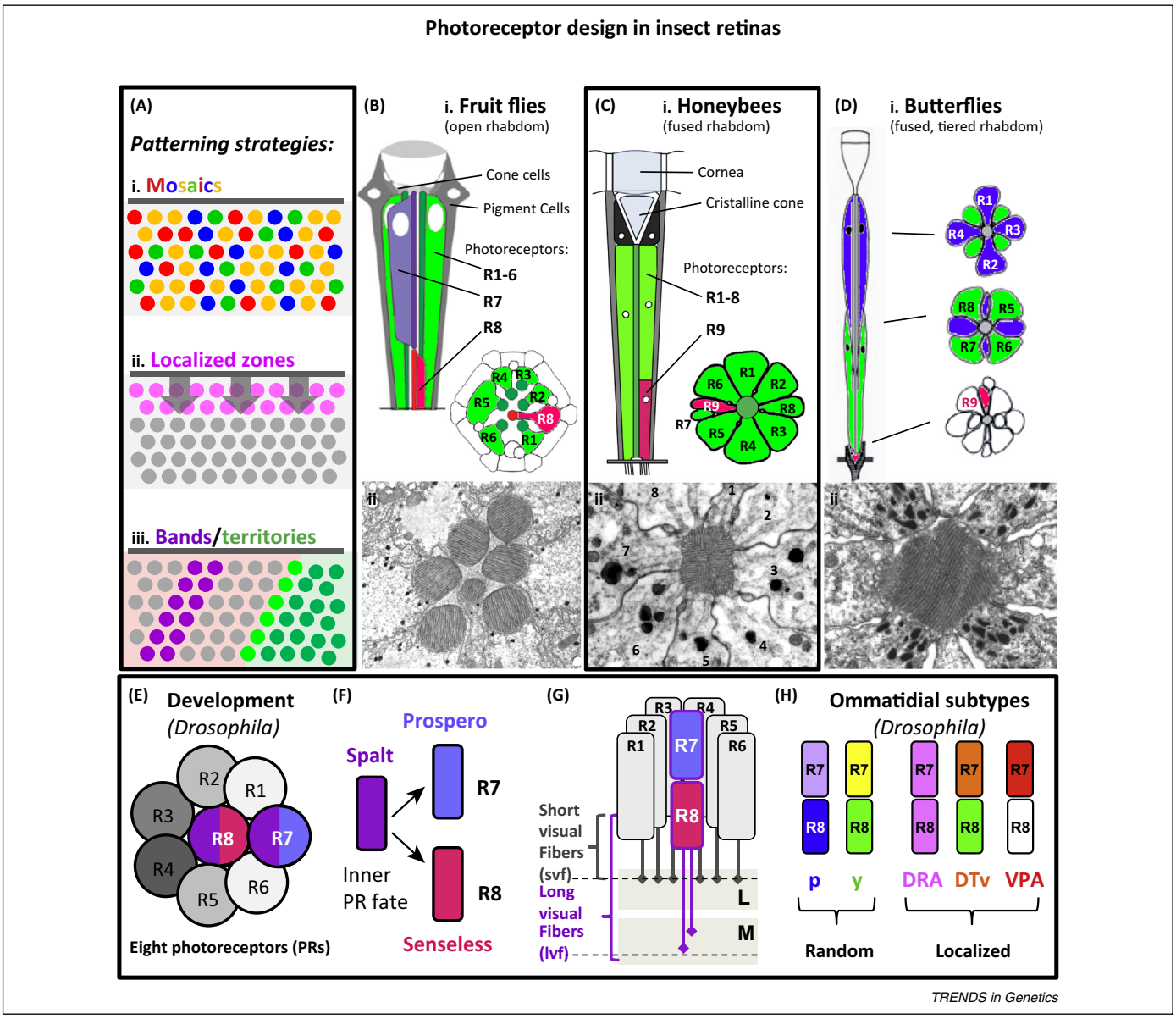


**Figure 1**. Insect retinal mosaics: common design principles, evolutionary, and molecular logic. **(A)** General patterning strategies common to many retinas. (i) Stochastic specification of retinal units within the epithelium creates a retinal mosaic. (ii) Localized specification of marginal units in response to factors emanating from adjacent, non-retinal tissue (gray line), in response to short-range signals (gray arrows). (iii) The specification of stripes or bands of retinal units can occur at compartment boundaries (light green) or within an otherwise seemingly homogeneous retinal field (purple). In some cases, all retinal units located inside a given compartment show the same, specific specializations (e.g., dark green). **(B–D)** Examples of evolutionary variation between insect ommatidia. (B) The fruit fly ommatidium (*Drosophila melanogaster*) contains eight neuronal photoreceptor cells (PRs), as well as non-neuronal cone cells and pigment cells. Six PRs span the entire thickness of the retina (termed R1–6, green), while R7 (blue) and R8 (red) are situated on top of each other. Inset: light-gathering organelles (rhabdomeres) of all eight PRs are separated from each other (dark-green and red circles) creating an 'open rhabdom'. (ii) Electron micrograph showing the open rhabdom structure with 'inner PRs' R7 and R8 located in the center (sectioned at the level of R7). (C) (i) the ommatidia of a honeybee worker (*Apis mellifera*) contain nine PRs, eight of which span the entire retina (R1–8, green), while the shorter cell R9 is always found basally (red). Inset: rhabdomeres of R1–9 are not separated ['fused rhabdom', labeled in the electron micrograph section in (ii)]. (D) (i) the ommatidia of many butterflies, such as the swallowtail *Papilio*, contain nine PRs, four of which are located in the distal retina (R1–4, blue), while four are found in the basal half (R5–8, green). Therefore, the PRs in butterfly ommatidia are tiered. Note that the very small cell R9 is always found at the base of the ommatidium (red), with little contribution to the rhabdom. Insets: the fused rhabdom of *Papilio* at three different levels, illustrating the tiered design. (ii) Transmission electron microscopy of a section through an ommatidium from the butterfly *Anthocharis* (Pieridae). **(E,F)** Developmental specification of ommatidial cell types in *Drosophila*. (E) Eight-cell cluster from wandering third-instar larvae. After initial recruitment from an undifferentiated pool of progenitors, PR cell fates are specified through combinatorial expression of transcription factors, such as Spalt (purple) + Senseless (red) in the case of R8, or Spalt + Prospero (blue) for R7. (F) Expression of Spalt in 'inner PRs' is crucial for their specification into R7 and R8 via Prospero and Senseless, respectively. **(G)** 'Inner PRs' R7 and R8 terminate in a deeper level of the brain, with long visual fibers (Lvf) projecting to the medulla (M) neuropil. Outer PR R1–6 have short visual fibers (Svf) terminating in the lamina (L) neuropil, thereby connecting to distinct postsynaptic partners. **(H)** Based on the molecular (and in some cases morphological) criteria of inner PRs, *Drosophila* ommatidia can be subdivided into five subtypes, which are discussed in the main text. Reproduced from [120] (Bi); [39] (Bii), (Cii); [9] (Di); [121] (Dii).

classical model organisms such as higher flies (Diptera, e.g., *Drosophila*), honeybees (Hymenoptera), and butterflies (Lepidoptera) exemplifies this problem (Figure 1B–D): while six 'outer PRs' called R1–6 span the entire thickness of the retina in the open rhabdom of *Drosophila*, the two 'inner PRs' occupy the center, with R7 located distally and R8 proximally (Figure 1B; reviewed in [7]). In honeybees, eight PRs span the entire thickness of the retina in a fused rhabdom, with one additional small PR located proximally (R9) that contributes little to the rhabdom (Figure 1C [8]). The proximodistal separation of PRs becomes more extreme in some butterflies, where two groups of four PRs each

contribute rhabdomeric microvilli in either the proximal or the distal side of the rhabdom. This 'tiered' butterfly retina is completed by a small proximal cell (R9), similar to honeybees (Figure 1D; reviewed in [9]). Although it seems reasonable to assume R9 in both honeybees and butterflies shares homology to *Drosophila* R8 cells (see below), no claims can easily be made about each of the eight other PRs [10,11].

A powerful system for the standardized numbering of insect PRs has recently been proposed [10], taking advantage of the well-understood specification of PR subtypes in a developing *Drosophila* ommatidium [10,11]. During larval stages, *Drosophila* PRs are recruited in a stereotypical order (R8 first, followed by R2/R5, R3/R4, then R1/R6, and finally R7; Figure 1E), which is deeply conserved and thereby allows identification of their homologous counterparts in other species [10–13]. Furthermore, developing *Drosophila* PR subtypes also express a well-characterized combination of transcription factors, defining their fate within the ommatidium (Figure 1E [14]). Some of the 'outer PRs' fall into pairs expressing identical combinations of transcription factors [such as Bar and Seven up (Svp) in R1 + R6, Rough in R2 + R5, and Rough + Svp in R3 and R4], and Svp expression in PRs may be conserved between species [13]. In *Drosophila*, both R7 and R8 are distinguished by the expression of the transcription factor Spalt, which is both necessary and sufficient for expression of inner PR markers [15,16]. The fates of R7 and R8 are then separated by two additional transcription factors: Senseless (Sens), expressed in R8, and Prospero (Pros) in R7 (Figure 1F [16–19]). Hence, the expression of markers such as Spalt, Sens, and Pros, which are expressed in PRs throughout the adult stages, could provide a more accurate way of identifying the homology of insect PRs [20,21]. Finally, *Drosophila* R7 and R8 can also be identified based on the length of their axons, which project to a deeper level of the optic lobe, called the medulla, whereas R1–R6 have short visual fibers that stop in the lamina (Svf). (Figure 1G [22]) PRs with 'long visual fibers' (Lvf) have been reported in many different insects [23], yet in most cases tracing the axon path is challenging and the identity of Lvf PRs within the ommatidium could not be defined. Instead, recent studies have focused on comparing Rhodopsin expression of Svf and Lvf PRs between species [24]. As we discuss below, Rhodopsin expression in R7 and R8 creates at least four ommatidial subtypes in *Drosophila*, distributed either stochastically or localized (Figure 1H [25,26]). Recent characterization of Rhodopsins in other insects now allows a more detailed comparison.

## Striking similarities between the stochastic retinal mosaics of different insects

The insect retinal mosaic is best understood in *Drosophila*, where differential expression of four Rhodopsin genes creates a mosaic of two stochastically distributed ommatidial subtypes (Figure 2A [5,25]). While outer PRs R1–6 in both subtypes express the broad-band Rhodopsin Rh1 (a Blue/Green Rhodopsin which associates with a UV-sensitizing pigment), 'pale' ommatidia express UV-sensitive Rhodopsin Rh3 in R7 and Blue Rh5 in R8, whereas 'yellow' ommatidia contain another UV Rhodopsin in R7 (Rh4) and a Green Rhodopsin in R8 (Figure 2B,C [27–29]). The subtype choice originally occurs in R7 through stochastic expression of the transcription factor Spineless exclusively in 'yellow' R7 cells (yR7) [30]. Spineless directly activates expression of Rh4 and represses the 'pale' fate in R7, also preventing the induction of the 'pale' fate in the underlying R8 cell, which is induced by an unknown signal from pR7 [29,31]. It was recently shown how the complex interplay between activator and silencer sequences from two *spineless* alleles results in the stable choice in R7 cells (Figure 2D [32]). Interestingly, 'pale' and 'yellow' ommatidia occur in an uneven ratio (35:65) that is conserved among higher flies [33]. How this bias is achieved remains unclear, as is its functional significance for the behavior of the animal.

Characterization of the honeybee Rhodopsin genes revealed striking similarities with the stochastic retinal mosaic of *Drosophila*, despite considerable differences in ommatidial organization [34]. Expression of UV-, Blue-, and Green Rhodopsins form three stochastically distributed ommatidial subtypes (termed I, II, and III; Figure 2E). Using the homology-based standardization of PRs, the three Lvf PRs of honeybees can be classified as two distal R7-like cells and one proximal R8 homolog [10]. As in *Drosophila*, 'outer PRs' with Svf always express the same long-wavelength Rhodopsin, while the R7-like cells choose stochastically between expression of the UV or Blue Rhodopsin [34]. Given its small size, the R8-like cell (R9) is difficult to characterize, yet it most likely expresses the long-wavelength Rhodopsin [34]. Fascinatingly, the overall occurrence of UV versus Blue R7-like cells is approximately 68:32 and, therefore, strikingly similar to the yR7:pR7 ratio in *Drosophila* (65:35). The uneven distribution of the three honeybee subtypes then amounts to 44% Type I (UV/B), 46% Type II (UV/UV), and 10% Type III (B/B) (Figure 2F). Therefore, it appears that two seemingly different retinal mosaics could be shaped by variations of the same molecular program using factors that are evolutionarily conserved between species.

Among insects, butterflies manifest some of the most complex stochastic retinal mosaics (reviewed in [9,35,36]) (Figure 2G). In the swallowtail *Papilio*, functional recordings revealed as many as six individual classes of PRs [9], but the genome appears to only contain five Rhodopsins: UV-, Blue- and three long-wavelength forms [9,35]. As in many other butterflies, their expression patterns in R7-like Lvf PRs (called R1 and R2 in butterflies) are strikingly similar to those in honeybees (Figure 2F): three stochastically distributed subtypes (I, II, and III) with stochastic choice between UV and Blue Rhodopsins, while the proximal R8-like cell (R9) co-expresses two of the long-wavelength Rhodopsins [9,35]. However, exactly 50% of R7-like cells express either UV- or Blue Rhodopsins, resulting in a subtype distribution of 50% Type I (UV/B), 25% Type II (UV/UV), and 25% Type III (B/B). Thus, whatever mechanism shapes the uneven ratio of three ommatidial subtypes with varying Rhodopsin expression in honeybees, in analogy to Spineless function in flies, is not found in this papillionid butterfly. It must be noted that duplication of the gene encoding Blue Rhodopsin in *Lycaenidae* led to an even more complex Lvf mosaic that would require an additional level or regulation (reviewed in [36]). The second

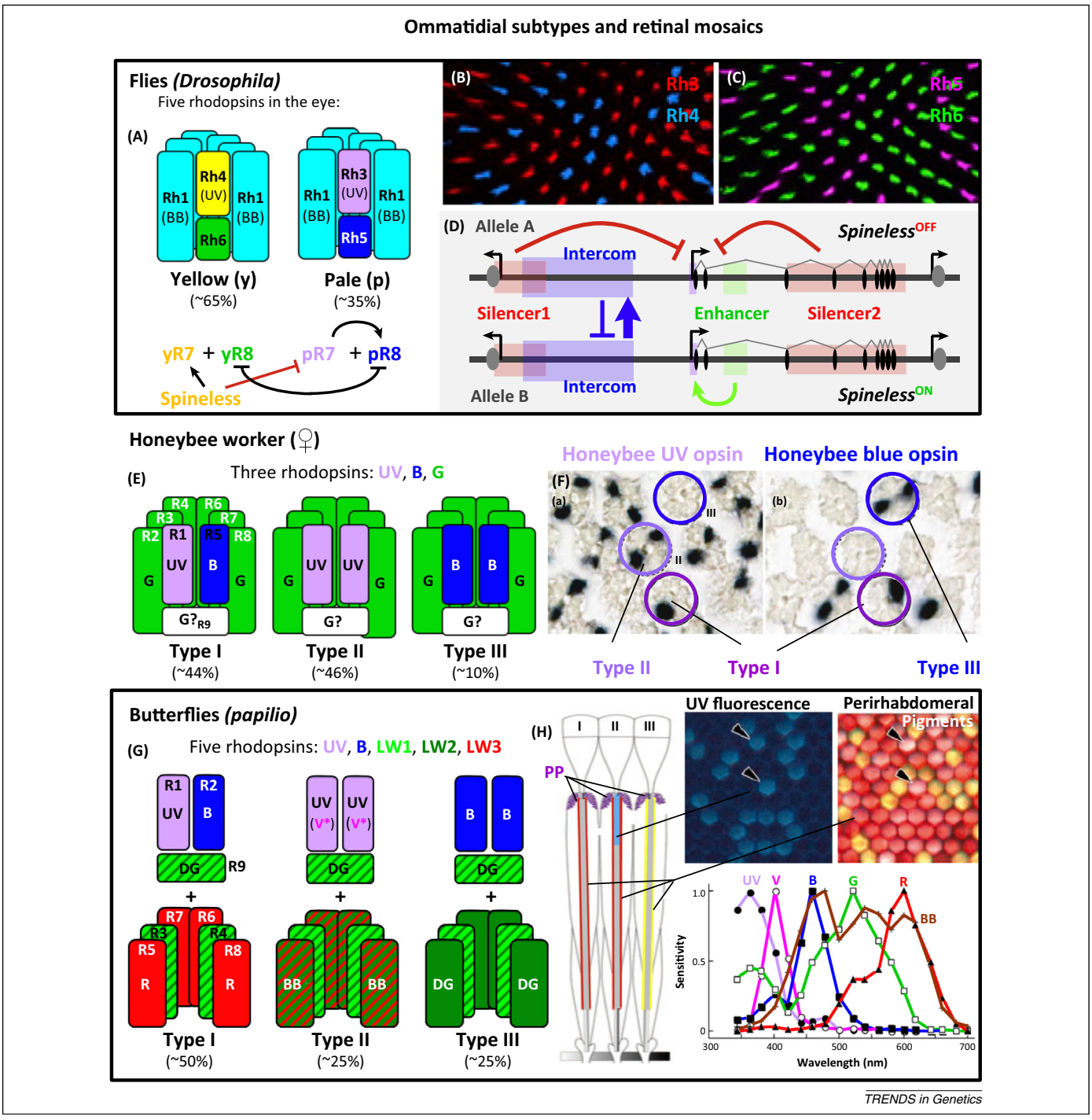


**Figure 2**. Common features and differences between ommatidial mosaics in different insects. **(A)** The *Drosophila* retina contains two ommatidial subtypes, named 'yellow' and 'pale', which are distributed stochastically in an uneven ratio (65:35). They differ in the Rhodopsins expressed in R7 [choice between UV opsins Rh3 and Rh4] and R8 (Blue opsin Rh5, or Green opsin Rh6), thereby creating a mosaic of chromatic sensitivities. The subtypes are first defined in R7 cells by stochastic expression of the transcription factor Spineless in the 'yellow' R7 subtype, where it represses the 'pale' fate. Only 'pale' R7 cells instruct underlying R8 cells to acquire the same subtype fate, while 'yellow' R8 cells choose their fate by default. A cellular signal transduction pathway maintains the chosen subtype fate in R8 cells by mutual repression. Abbreviation: BB, broad-band Rhodopsin associated with a UV-sensitizing pigment. **(B)** Stochastic distribution of 'pale'/'yellow' rhodopsins Rh3 (red) and Rh4 (blue) in the R7 layer of the adult *Drosophila* retina. **(C)** Similar stochastic distribution of Rh5 (purple) and Rh6 (green) in the R8 layer. **(D)** Molecular mechanism driving stochastic expression of *spineless* in R7 cells. Activating (green) and repressing (red) *cis*-regulatory elements determine on–off expression. Furthermore, interchromosomal long-range communication (via 'intercom', blue) modulates the frequency of expression, and coordinates expression state between alleles. As a result, both alleles are expressed in the same random subsets of R7 cells. Spineless encodes a PAS-bHLH transcription factor that then activates 'yellow'-specific downstream genes. **(E)** The retinal mosaic of the honeybee worker (*Apis mellifera*) contains three stochastically distributed subtypes, named I (44%), II (46%), and III (10%). Similarly to *Drosophila*, the mosaic is defined by differences in Rhodopsin expression in two PRs with long visual fibers (Lvf), R1 (choice between UV or B/blue) and R5 (UV or B/blue). The identity of R9 remains obscure due to its small size. Similar to *Drosophila*, all PRs with short visual fibers (Svf) express the same long-wavelength Rhodopsin (G/green). (Lvf PRs are shown in the center of the ommatidial schematic, following the nomenclature of [10]). **(F)** Stochastic expression of honeybee rhodopsin genes in the adult retina visualized by *in situ* hybridization against UV (left) and blue opsins (right). **(G)** The ventral retina of the swallowtail butterfly *Papilio* also contains three stochastically distributed ommatidial subtypes, named I (~50%), II (~25%), and III (~25%). As in the honeybee, distal PRs with Lvfs (R1 and R2) choose between expression of UV and Blue Rhodopsins. The basal PR always co-expresses two long-wavelength Rhodopsins (LW1 and LW2). Unlike in *Drosophila* and honeybees, the mosaic is not uneven and extends to PRs with Svf: two cells always co-express the same two long-wavelength Rhodopsins as PR R9, while the remaining four cells manifest different combinations of long-wavelength Rhodopsins. The

*(Figure legend continued on the bottom of the next page.)*

striking feature of *Papilio* lies in the extension of stochastic mosaic Rhodopsin expression into the Svf 'outer PRs': only two Svf cells are identical between all three subtypes (expressing one long-wavelength Rhodopsin dorsally, or co-expressing the same two long-wavelength Rhodopsins as the basal Lvf cell R9, ventrally). The remaining four cells either choose between two long-wavelength Rhodopsins (Type I/III), or co-express them (Type II). Fascinatingly, this choice by Svf PRs is therefore linked to the UV/B subtype decision in the R7-like cells, while the third Lvf cell R9 seems invariable. Perhaps the same signaling pathway that coordinates expression of R7 and R8 Rhodopsins in *Drosophila* pale and yellow ommatidia [31,32] is reused in butterfly Svf cells. Interestingly, the Svf mosaic is absent in the nymphalid Monarch butterfly (*Danaus plexippus*), where all six Svf cells express the same long-wavelength Rhodopsin, a situation that has been proposed to be ancestral [36,37]. The final unique feature of *Papilio* lies in the mosaic expression of additional pigments, such as the fluorescent 3-hydroxyretinol in Type II ommatidia (shifting sensitivity of R7-like cells from UV to violet), and the 'perirhabdomeral pigments' expressed by Svf PRs (red pigment in Type I/II and yellow pigment in Type III) [9,35]. Hence, expression of their biosynthetic enzymes must also be co-regulated with rhodopsin expression. Together with a purple pigment expressed in R7-like cells of all three ommatidial subtypes, the combined mosaic expression of *Papilio* pigments shapes the complex chromatic sensitivity curves recorded for butterfly PRs (Figure 2H), which in turn serve as the basis for their exceptional color vision system [38].

## The evolution of localized specializations with defined functions

A well-understood example of locally specified retinal units in many insect species are the ommatidia containing polarization-sensitive PRs in the 'dorsal rim area' (DRA) (reviewed in [39]). In many insect PRs, polarization sensitivity is abolished by continuously twisting the rhabdomeres, thereby reducing sensitivity to specific e-vector orientations [39,40]. Behavioral and physiological data support a role of the specialized DRA in detecting the celestial polarization pattern for navigation [40]. In *Drosophila*, DRA ommatidia form a narrow band of one to two ommatidial rows along the dorsal head cuticle (Figure 3A [41–43]). Their Rhodopsin expression in R7 and R8 is unique, because both express the UV Rhodopsin Rh3 [42–45]. Their morphology is also specialized: untwisted rhabdomeres of R7 and R8 with an enlarged diameter form polarization detectors oriented at orthogonal angles (R7 versus R8 [46]) that allow the animal to detect the e-vector orientation of linearly polarized light (Figure 3B; reviewed in [39,40]). Localized specification of *Drosophila* DRA ommatidia results from combining positional information provided by 'dorsal selector genes' in the retina (the transcription factors of the *Iroquois* complex, *Iro-C*) with a diffusible signal emanating from the head cuticle all around the eye (Wingless, Wg; Figure 3C). As a result, the homeodomain transcription factor Homothorax (Hth) becomes expressed in DRA R7 and R8 cells (Figure 3D), where it is both necessary and sufficient to induce DRA fate by modulating the retinal transcriptional network in inner PRs together with Spalt [43,44,47,48].

The recent characterization of Rhodopsin genes from two prominent polarization vision model species, crickets and desert locusts, has led to important new insight into the evolutionary mechanisms shaping insect retinal mosaics [49,50]. As in flies, the DRA ommatidia of crickets (*Gryllus bimaculatus*) show specialized Rhodopsin expression (Figure 3E): five PRs (one Lvf cell and four Svf) all express a blue-sensitive Rhodopsin, while the small proximal PR (R8) expresses a UV Rhodopsin (the two remaining Svf cells do not contribute to the rhabdom and their Rhodopsin expression remains unclear) [49,51]. Interestingly, the five blue-sensitive receptors form two groups of untwisted rhabdomeres, with microvilli of R7 (Lvf) orthogonal to those of R1,2,5, and 6 (Svf) (Figure 3F [51]). This Rhodopsin pattern fits well with behavioral and electrophysiological data describing Blue receptors with high polarization sensitivity in the DRA [52–55], yet the function of the UV-expressing proximal cell in DRA ommatidia remains elusive because it does not seem to be a functional part of the DRA polarization detection system. Unlike in flies, a cricket Homothorax-like factor regulating DRA fate including Rhodopsin expression must be expressed in both Lvf and Svf PRs close to the dorsal head cuticle, probably by uncoupling its regulation from an Lvf-specific factor such as Spalt.

In contrast to the predominantly blue-sensitive DRA, the central part of the cricket retina exhibits strong expression of the long-wavelength (Green) Rhodopsin, while Blue Rhodopsin is completely absent [49]. Based on previous electrophysiological studies, the R7-like Lvf PR is most likely UV-sensitive, while the Svf/Lvf nature, as well as the Rhodopsin expressed in the small proximal cell, remain obscure [51,53]. So far, there is no evidence of a stochastic ommatidial mosaic in the central part of the cricket retina, defined by Rhodopsin choice in the Lvf cell. The remaining six Svf PRs are most likely green sensitive [49,51,53] (their PR identities could not always be assigned, as originally defined in [56]) (Figure 3G). The presence of a 'ventral band' of ommatidia with altered Rhodopsin expression in cricket (Blue and Green Rhodopsins) was unexpected [49]. Its molecular specification and behavioral importance remain unknown, yet similar retinal specifications exist in other insect species and are discussed below.

An even more extreme molecular specialization of DRA ommatidia was recently described in the desert locust (*Schistocerca gregaria*) [50], another classical polarization vision model [57,58]. In the DRA, the blue-sensitive Rhodopsin is expressed by all PRs (Figure 3H), a situation reminiscent of Monarch butterflies, where a UV Rhodopsin is expressed in most DRA PRs (R1–8), including Svf and

spectral sensitivity of most of these cell types is further modulated by the presence of additional pigments (V*, violet; R, red-sensitive; BB, broad band; DG, double-peaked green). **(H)** Schematic of *Papilio* type I–III ommatidia depicting the additional pigments: while all three subtypes contain purple pigment (PP) granules distally in R1 and R2, they differ in perirhabdomeral pigment content: red (type I and II) or yellow (type III), resulting in different coloring when back-illuminated (inset, top right). Finally, only type II ommatidia contain 3-hydroxyretinol, visible by UV-induced fluorescence (inset, top center). This pigment shifts the sensitivity of type II R1 and R2 UV PRs towards violet light [see spectral sensitivities at bottom of (H)]. Reproduced from [31] (B), (C); [32] (D); [34] (F); [9] (H).

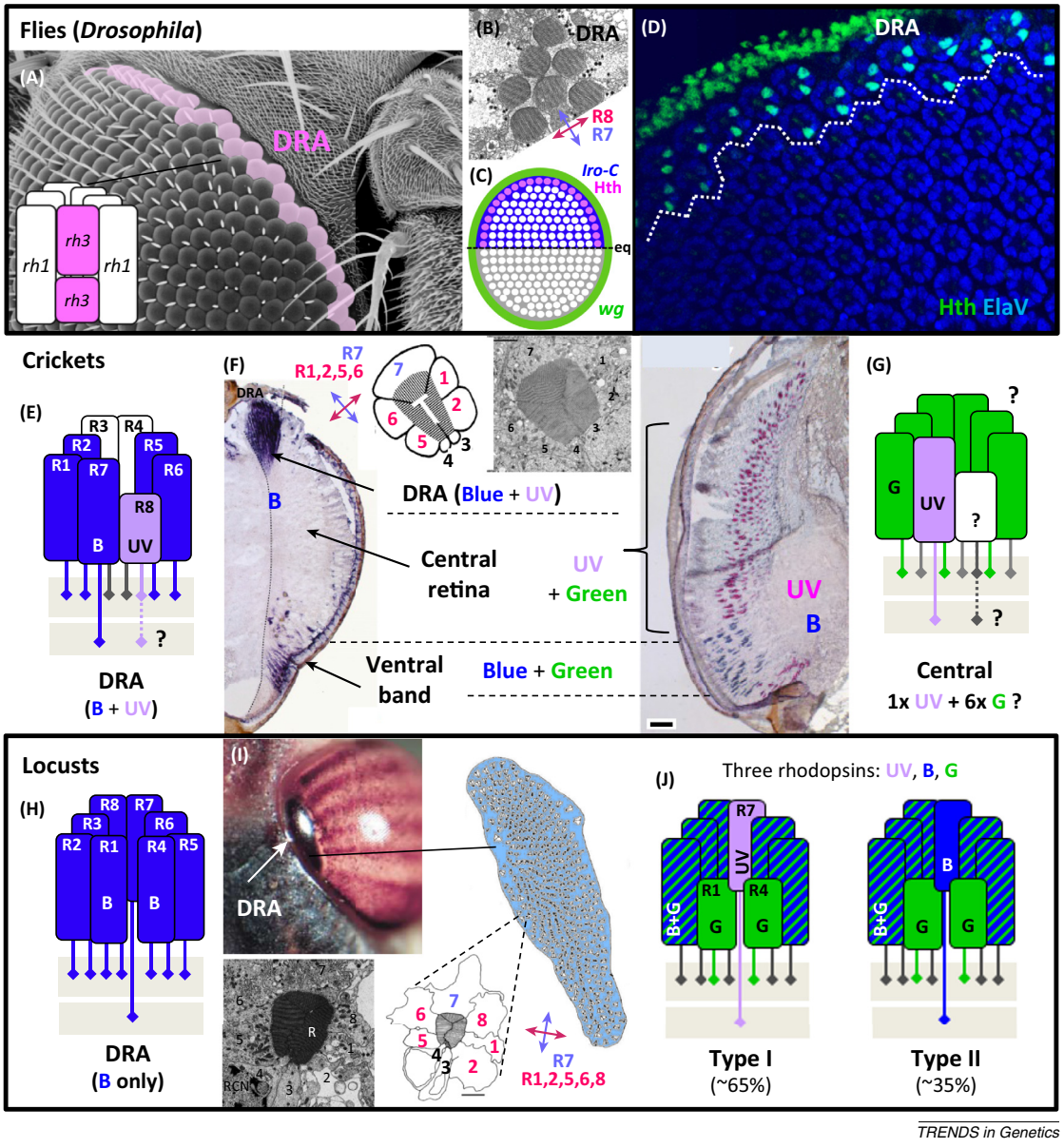


**Figure 3**. Localized specification of dorsal rim ommatidia of flies, crickets, and desert locusts. **(A)** Scanning electron microscopy (EM) with the approximate location of one to two rows of specialized ommatidia in the dorsal rim area (DRA) in *Drosophila*. Inset: only DRA ommatidia express the UV Rhodopsin Rh3 in both R7 and R8. **(B)** In the DRA, the diameter of R7 and R8 PR rhabdomeres is enlarged (compare with Figure 1B). Furthermore, rhabdomeric microvilli are untwisted and oriented at an angle of 90° (symbolized by the double-headed arrows). **(C)** During development, the DRA fate is induced by combining positional information provided by the dorsal selector genes of the Iroquois complex (Iro-C, blue) and high levels of Wingless signaling (Wg, green), which induce an unknown diffusible signal emanating from the adjacent head tissue all around the eye. **(D)** The homeodomain transcription factor Homothorax specifically marks R7 and R8 nuclei of DRA ommatidia (Hth shown in green, co-labeled with the pan-neuronal marker Elav in blue), where it is both necessary and sufficient to induce the DRA fate. **(E)** Summary of Rhodopsin expression in cricket (*Gryllus bimaculatus*) DRA ommatidia. As in *Drosophila*, cricket ommatidia contain eight PRs, at least one of which has long visual fibers (Lvf; identity of the proximal cell remains unclear). Five PRs in the DRA [Lvf + four short visual fibers (Svf)] express Blue Rhodopsin. The proximal cell expresses UV Rhodopsin, while two cells do not contribute to the Rhabdom (shown in white; Rhodopsin expression unknown). **(F)** *In situ* hybridizations visualizing the expression of cricket Rhodopsins in the adult retina. Note expression of blue opsin outside the DRA, only in a previously undescribed ventral band of ommatidia. Inset, top: summary and scanning EM of DRA morphology where two groups of PR forming orthogonally oriented, untwisted rhabdomeric microvilli: R7 versus R1,2,5, and 6 (white 'T' indicates orientation of the ommatidium). **(G)** Summary of Rhodopsin expression in cricket central ommatidia: the Lvf cell most likely expresses a UV Rhodopsin, while Rhodopsin expression in the proximal cell remains unknown. The six remaining Svf PRs most likely express the same Green Rhodopsin (exact PR identities could not be determined: question mark). **(H–J)** Summary of Rhodopsin expression in ommatidial subtypes in the retina of the desert locust (*Schistocerca gregaria*). (H) DRA ommatidia are dramatically specialized, with all PRs expressing only the Blue (B) Rhodopsin. (I) Specialized morphology of locust DRA ommatidia, where R7 forms untwisted rhabdomeric microvilli that are oriented at 90° to those of R1,2,5,6, and 8. (J) Outside the DRA, five Svf PRs always co-express blue (B) and green-sensitive (G) Rhodopsins, while two basal Svf cells express only the Green Rhodopsin (R1+R4). Additionally, a mosaic of two ommatidial subtypes exists: type I (65%) and type II (35%). Opsin expression in the only PR with long visual fibers (R7) defines the mosaic by choosing between UV (Type I: UV) and blue (Type II: Blue). Reproduced from [44] (A), (B); [47] (C); [49,52] (F); [60] (I).

Lvf PRs [37]. The exclusive expression of Blue Rhodopsin in the locust DRA was surprising, given that UV receptors of low polarization sensitivity had previously been described in the locust DRA [59], where DRA ommatidia form a fan-shaped array of detectors [60,61] (Figure 3I). Interestingly, in the main part of the locust retina, seven Svf PRs express a long-wavelength (Green) Rhodopsin, five of which also co-express Blue Rhodopsin, leaving two

proximal PRs (R1 and R4) that are exclusively green sensitive [50,62]). The remaining single Lvf cell (R7) expresses either a UV (Type I) or a Blue (Type II) Rhodopsin, thus defining two subtypes of ommatidium that are distributed randomly with a ratio that is almost identical to that reported in *Drosophila* (65% Type I, 35% Type II) (Figure 3J). Despite these important similarities, it must be pointed out that the recent demonstration of only one Lvf cell per ommatidium in locusts was different from that in flies, honeybees, and butterflies [23,62,63]. The functional implications of one versus two Lvf cells per ommatidium are not clear, yet the advantage of comparing a delayed signal propagating through a multi-synaptic Svf→Lamina pathway versus a faster Lvf channel into the medulla has been proposed for the locust DRA [62]. Taken together, the extreme molecular specialization of the DRA in both crickets and locusts is reminiscent of the Monarch DRA, while non-DRA ommatidia form potentially homogeneous territories in crickets, or two stochastically distributed subtypes in locusts.

## The generation of regional differences and their behavioral relevance

Beyond the localized specification of ommatidia in the periphery, defined territories of the insect retina can adopt a specific functional organization, for instance when forming acute zones with increased resolution [64]. Often, dorsal and ventral regions of the retina show important differences in morphology, physiology, and Rhodopsin expression [49,65,66]. In *Drosophila*, specialized ommatidia co-expressing both R7 Rhodopsins (Rh3+Rh4) are located in the dorsal-most third of the adult retina (Figure 4A) [67]. Molecularly, these ommatidia represent an unusual form of the 'yellow' subtype, in which expression of Rh3 has been de-repressed (DTy, 'dorsal third yellow' [31,67]) (Figure 4B). Similar Rhodopsin co-expression had been known in butterflies [68,69], and increasing evidence now suggests it is a common theme in insect retinas, rather than the exception [50,65–70]. The *Drosophila* transcription factors of *Iro-C* are expressed specifically in the dorsal half of the developing fly eye (Figure 4C), where they direct the development of dorsal structures and positioning of the equator of the eye [71–73]. Iro-C factors attenuate Spineless levels in the dorsal third, resulting in low levels of the repressor of Rh3, Defective proventriculus (Dve) [31]. As a result, Rh3 becomes derepressed, whereas Rh4 levels are unaffected by low Spineless levels (Figure 4D, [31]). Using this simple mechanism, a new class of ommatidia is created in the part of the eye facing the sky. However, the functional significance of DTy ommatidia remains unknown due to the lack of *Drosophila* ethological data. Existing behavioral and physiological data from other insects provide clues to the function of such dorsoventral territories.

Dragonflies serve as an attractive model for studying the chasing behavior of a flying predator that calculates an interception course to its prey (reviewed in [74]). During this complex behavioral task, the animal always fixates the prey with the dorsal half of its eye (Figure 4E). The dorsal eye of the dragonfly *Sympetrum* contains pronounced morphological specializations (Figure 4F): a yellow screening pigment in combination with a high frequency of blue receptors in dorsal *Sympetrum* ommatidia form a highly sensitive system that includes a fovea region with small inter-ommatidial angles perfectly adapted for the fast tracking of prey against the bright-blue sky [75,76]. Although no sexual differences have been reported for dragonflies, this blue-sensitive dorsal specialization is reminiscent of exclusive Blue- and UV Rhodopsin expression in the dorsal retina of the male butterfly *Lycaena rubidus* [66]. It should be noted that, in addition to specialized dorsal ommatidia, the dragonfly also has DRA ommatidia for the detection of polarized skylight [77]. Interestingly, other dragonfly behaviors depend on the ventral retina, such as the detection of water surfaces for establishing a territory (males) or oviposition (females) [78]. Physiological characterization of the ventral dragonfly retina using electroretinograms (ERGs) revealed a high frequency of Green receptors, further demonstrating the functional separation (Figure 4G [76]). In fact, intracellular recordings and recent sequencing of up to 30 different Rhodopsins from individual dragonfly species paint a complex picture of the ventral retinal mosaic with many different PR classes of different chromatic sensitivity, most likely due to co-expression of several Rhodopsins (UV, Blue, and Green), as well as additional pigments [79–81]. Hence, the retina is divided into two separate halves, each serving distinct behavioral tasks.

Another interesting example of dorsoventral segregation of retinal mosaic structure and function is the male honeybee drone, whose dorsal retina also mediates pursuit behavior (chasing the queen), while the hive entrance is approached through fixation with the ventral eye (Figure 4H [82]). Interestingly, expression of Rhodopsin transcripts changes drastically at the equator of the drone retina, [83], thereby resembling certain transcripts from dragonflies [81]. Most PRs in the dorsal half of the honeybee drone retina seem to express the Blue Rhodopsin, whereas the same cell types express the Green Rhodopsin in the ventral half, a situation similar to the ommatidia of the worker bee retina discussed above (Figure 4I). Some uncertainty remains about the Rhodopsins expressed by the Lvf cells in the specialized, blue-sensitive dorsal ommatidia of the drone (termed here '$DA_{drone}$'), but strong expression of the UV Rhodopsin suggests they are unusual Type II (or Type I) ommatidia with blue-sensitive Svf PRs [83]. Although most of the dorsal retina of the honeybee drone contains this specialized $DA_{drone}$ subtype, it remains unclear whether expression of UV and Blue receptors in the ventral retina leads to stochastically distributed ommatidial subtypes [83], as in the case of the worker bee (Figure 4J). In conclusion, the drone eye differs greatly from the worker bee both molecularly, as well as in size, shape, and ommatidia number [84]. Although drones do not participate in pollenating flights that require precise navigation, their eyes also contain DRA ommatidia [85,86]. Hence, in addition to patterning the DRA, factors such as Iro-C may be used in the honeybee drone to transform the entire dorsal retina into $DA_{drone}$ ommatidia serving another function. Interestingly, behavior experiments with honeybee workers have demonstrated differences in color discrimination tasks mediated by the dorsal versus the ventral halves of the retina

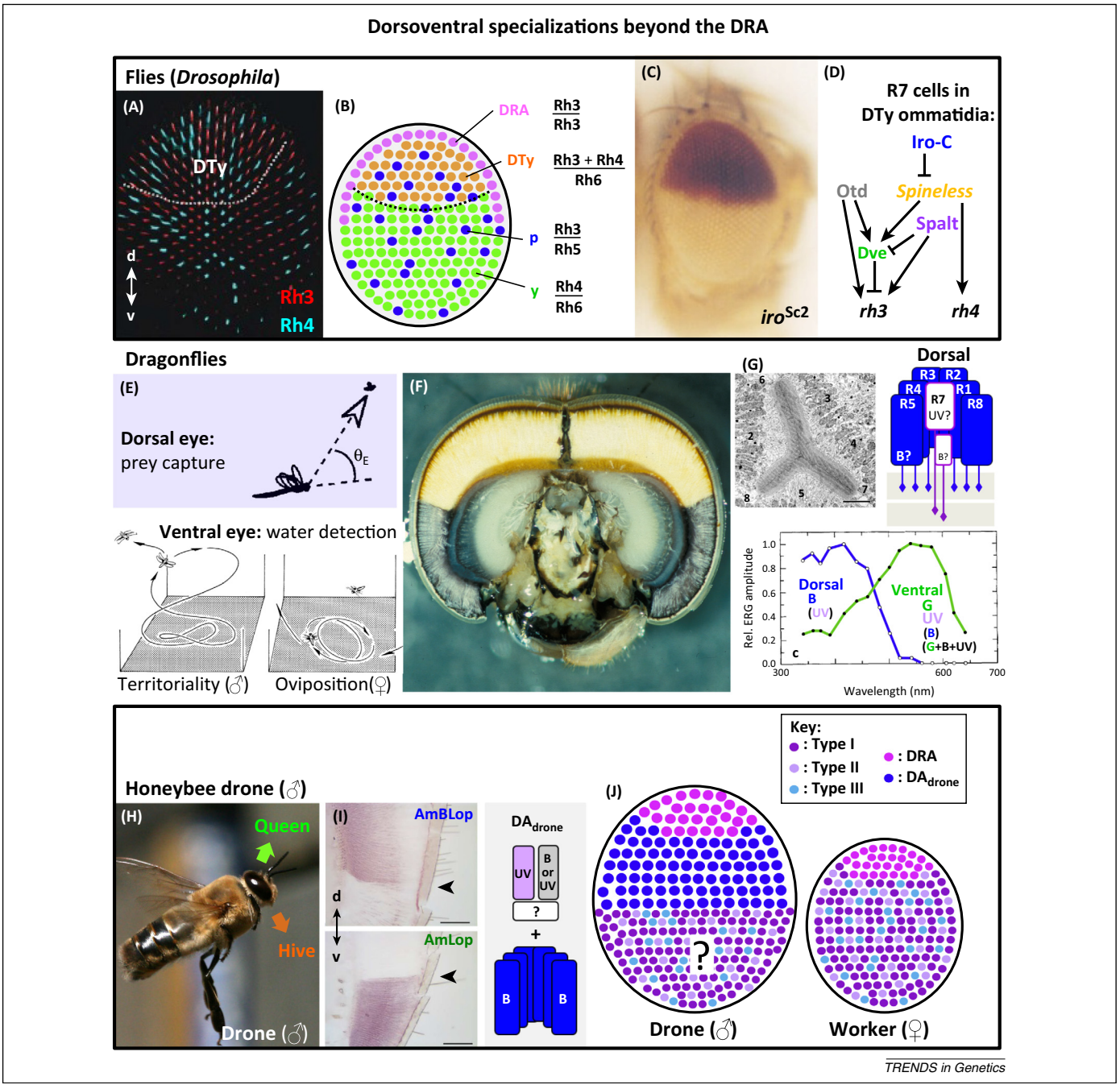


**Figure 4**. Molecular and morphological specializations in the dorsal retina. **(A)** In the dorsal third of the adult *Drosophila* retina, specialized 'yellow' ommatidia co-express both UV Rhodopsins, Rh3 and Rh4, creating a fourth ommatidial subtype (DTy). **(B)** Schematic summarizing the retinal mosaic in *Drosophila*, containing four ommatidial subtypes defined by unique Rhodopsin expression: pale (blue), yellow (green), DTy (orange), and dorsal rim area (DRA; pink). **(C)** Eye pigmentation of an enhancer trap element inserted in the Iroquois (*Iro-C*) complex of dorsal selector genes. **(D)** Summary of the transcription factor network regulating Rh3/Rh4 co-expression in R7 cells of DTy ommatidia. By modulating Spineless levels, Iro-C attenuates repression of *rh3* via the transcriptional repressor Defective proventriculus (Dve), while Rh4 levels remain unaffected. **(E)** Examples from dragonflies, for different visual behaviors mediated by the dorsal half of the eye (top: prey capture) versus the ventral half of the eye (bottom: detection of water surfaces as habitat or oviposition sites). **(F)** Section through a dragonfly (*Sympetrum*) eye demonstrating the obvious morphological differences between dorsal and ventral retina. Note the sharp boundary between dorsal retina (expressing yellow pigment) and ventral retina. **(G)** Summary of morphological and molecular features of the dorsal dragonfly retina. Top: ommatidia form fused rhadoms with eight PR cells, two of which have long visual fibers (Lvf; R6, R7). In the dorsal retina, the short visual fiber (Svf) PRs most likely all contain Blue Rhodopsin. Bottom: Electroretinogram (ERG) of the dragonfly eye shows prevalence of blue receptors in the dorsal half, whereas the ventral retina is mostly green sensitive. Electrophysiological studies point to a variety of cell types with different spectral sensitivities in the ventral eye, most likely co-expressing different Rhodopsins (UV, B, G, and UV+B+G), as well as additional pigments. **(H)** Photograph of a male honeybee drone, summarizing evidence of different behaviors that are mediated by the dorsal and ventral halves of the retina: chasing of the queen (dorsal) and approaching the hive (ventral). **(I)** Left: *in situ* hybridization of adult retinas of male honeybee drone visualizing dramatic expression of Blue Rhodopsin in the dorsal retina, whereas Green Rhodopsin is found in the ventral half. Note the sharp boundary in expression domains (black arrowhead). Right: schematic proposing an explanation for the specialized blue-sensitive dorsal ommatidia of the drone ($DA_{drone}$) ommatidial subtype found in the dorsal drone eye (compare to Figure 2E). **(J)** Summary of differences in the retinal mosaic between drones (left) and worker honeybees (right). Note the difference in overall retina size, facet diameter, and ommatidia number. While specialized $DA_{drone}$ ommatidia occur only in the dorsal retina of drones, it remains unclear whether three stochastically distributed subtypes exist ventrally (question mark). Alternatively, UV and Blue Rhodopsins could be organized uniformly in Type I ommatidia. Reproduced from [67] (A); [122] (C); [74,78] (E); [39,76] (G); [83] (I).

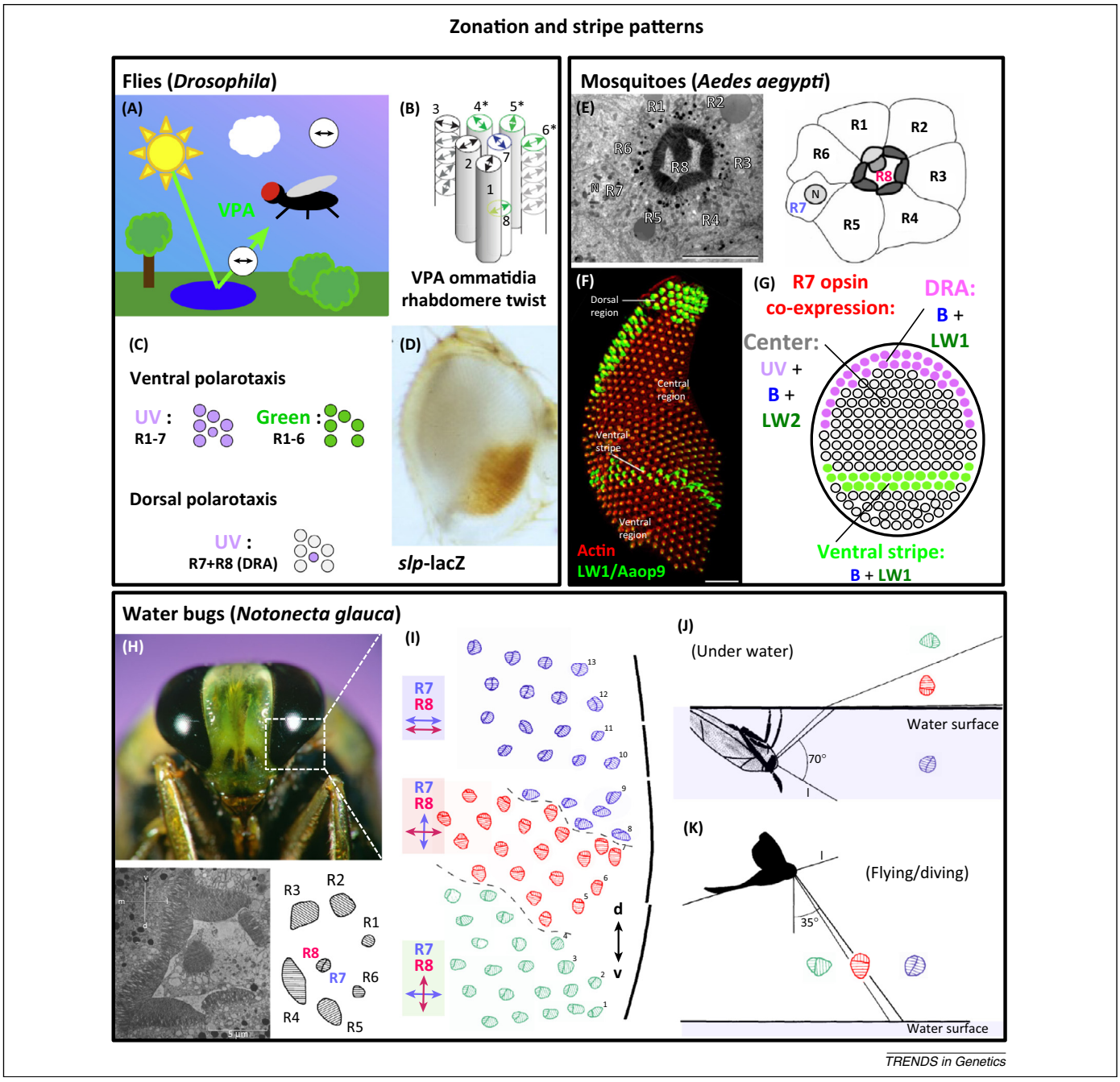


**Figure 5**. Retinal specializations in the ventral half of the insect retina. **(A)** Visual stimuli influencing the navigational decisions of flying insects, such as *Drosophila*: the sun, sky polarization (double-headed arrow), chromatic gradients in the sky, landmarks (trees, bushes), optic flow, and polarized reflections from water surfaces. **(B)** Morphological reconstruction of photoreceptor (PR) rhabdomere twist in the ventral retina using serial electron micrograph (EM) sections of R1–6 and R7 revealed a reduced twist of R7 and rhabdomeres in three out of the six short visual fiber (Svf) PRs (R4–6, symbolized by the green, double-headed arrows. **(C)** Behavior in response to ventral stimuli is strongly dependent on PRs with Svfs (R1–6), both using UV (R1-6+R7) and green stimuli (R1–6). R7 cells are involved in UV vision only. By contrast, dorsal polarotaxis relies on DRA R7 and R8 alone. **(D)** Ventral eye pigmentation from an enhancer trap inserted into the *sloppy paired* (*slp*) locus. **(E)** PR morphology of the mosquito *Aedes aegypti*, the vector of several pathogens dangerous to humans. **(F)** Whole-mounted adult mosquito retina stained for the long-wavelength Rhodopsin LW1/Aaop9 (green) expressed in R7 cells. Note expression in the dorsal region, as well as in a ventral stripe of ommatidia. **(G)** Schematic summary of mosquito opsins co-expressed in R7 cells of nonoverlapping regions: 'dorsal region' and the ventral stripe co-express the same Blue and Green Rhodopsin, while the central region in between co-expresses three Rhodopsins (UV+B+G), resulting in broad band sensitivity. **(H)** Summary of the visual system of the backswimmer *Notonecta glauca*, a water bug (Hemiptera). Top: photograph of the *Notonecta* eyes. Bottom: EM and drawing of a *Notonecta* ommatidium. Note that the rhabdomeres of R7 and R8 are fused, while R1–6 form an open rhabdom. **(I)** Zonation of the retina of *Notonecta* in the ventral eye region (only central rhabdomeres are shown). The rhabdomeres of R7 and R8 of dorsal-most ommatidia in this region (shown in blue) are aligned in parallel, as in the rest of the eye (G). R7 and R8 rhabdomeres in the more ventral ommatidia (shown in red and green) are aligned perpendicular to each other. Within a narrow stripe (in between broken lines), the central rhabdomere pairs are rotated so they align with the dorsoventral axis (red ommatidia), rather than in the mediolateral direction, as the ventral-most ommatidia (shown in green). **(J)** Behavioral significance of the zonation within the ventral *Notonecta* retina: when hanging below the water surface, ommatidia within the ventral band (shown in red) view the bright region of the water surface, and are perfectly tuned for detecting vertical contours of objects floating on the water surface. **(K)** When the animal is flying, ventral ommatidia with perpendicularly arranged rhabdomeres (red and green) serve as specialized detectors for water surfaces, which reflect horizontally polarized light. Reproduced from [123] (B); [96] (D); [100] (E); [101] (F). Part (H) is reproduced with kind permission from the photographers © Heidi and Hans Koch.

[87]. This situation might be more similar to *Drosophila*, where DTy ommatidia could also influence the color vision system of the animal. Existing behavioral color vision assays should be used to address this problem [88–90]. Taken together, molecular insights from *Drosophila* and other species (behavior, morphology, and physiology) can be combined to reveal how the insect retinal mosaics are adapted to the needs of the animal.

## Emerging design challenges: retinal bands and stripes

As discussed above, some insects have a specialized ventral retina with discrete morphological and functional features [49,75]. *Drosophila* have been shown to detect linearly polarized light with the ventral eye [46,91], a behavior that is independent of the DRA, which is only used for the detection of celestial stimuli [46,92]. Such ventral polarization vision can enable the detection of water surfaces, which reflect horizontally polarized light (Figure 5A; reviewed in [93]), resulting in attraction or avoidance behaviors [94,95]. The 'ventral polarization area' (VPA) mediating this behavior in *Drosophila* remains incompletely characterized [46]. Unusual ommatidia with untwisted R7 rhabdomeres as well as three Svf cells (R4–6) with reduced rhabdomeric twist exist in the ventral eye (Figure 5B), providing a sufficient anatomical substrate for detecting polarized UV light (using R7), or green light ventrally (using R4–6) [46] (Figure 5C). It is not known how the VPA is specified in *Drosophila*. However, genomic enhancers from *Drosophila* specifically driving expression in the ventral retina exist, for instance from the *sloppy paired* (*slp*) locus, which encodes two transcription factors [96] (Figure 5D). Therefore, it is possible that such factors modulate the retinal mosaic in a way similar to Iro-C in the dorsal eye [31,67].

Given that a good understanding of the ethology of *Drosophila* is lacking [97], turning to mosquitoes promised new insight into the interaction between visual behavior and the semi-aquatic life style of these insects [98,99]. Unlike PRs in its distant cousin *Drosophila*, PRs in *Aedes aegypti* form a fused rhabdom, and express at least five Rhodopsins (Figure 5E). Recent investigation of the retinal mosaic from *Aedes* revealed several surprising findings [100–102]: first, there is no apparent stochastic mosaic of ommatidial subtypes, based on Rhodopsin expression in R7 cells. Second, the four R7 Rhodopsins (UV, Blue, and two long-wavelength) are always co-expressed, yet in different combinations, between different regions of the eye. Third, the R7 cells in the dorsal-most *Aedes* ommatidia co-express two non-UV Rhodopsins (Blue and Green), making their chromatic sensitivity more similar to the DRA ommatidia of crickets and locusts (Figure 5F; reviewed in [39]). Finally, in an unusual stripe of ommatidia within the ventral half of the eye reminiscent of the 'ventral band' from crickets, R7 co-expresses the same Blue and Green Rhodopsins as in the dorsal-most ommatidia (Figure 5G). Interestingly, such elongated horizontal zones have been described predominantly in the eyes of arthropods living in flat environments, such as water surfaces or deserts (reviewed in [4]). Therefore, the 'ventral stripe' of mosquitoes and the ventral band of crickets could serve a similar function as the VPA in *Drosophila* [46]. Just like a larger ventral territory, it remains a fascinating question how such a ventral stripe is patterned during development because positional cues provided by Iro-C (and Slp-like factors) seem insufficient to explain such a narrow specialization.

Probably the best-understood aquatic insect is the hemipteran 'back swimmer' (*Notonecta glauca*), which can be found flying or diving into ponds, where they often hang under the water surface (Figure 5H). The ommatidial design of this species is interesting in that Svf PRs form an open rhabdom, while the rhabdomeres of two Lvf inner PRs are fused together [103,104]. The ventral *Notonecta* retina exhibits a stunning organization into three separate zones in which the microvilli orientations of R7 and R8 cells change rather abruptly (Figure 5I [103]). A correlation of this zonation with the body posture of the animal during flight or while hanging under water revealed that the visual angles of each zone are perfectly tuned for vision under water, at the water surface, or in the air, respectively: given the polarization of reflected light, the different microvillar orientations within the respective zones ensure maximal visual performance [103,105,106]. This example represents a satisfying link between ventral retinal design and the behavioral challenges an animal meets. It could serve as a starting point to better understand the ventral specializations of other insects discussed above. Although little is known about the *Notonecta* retinal design outside this specialized ventral region, a stochastic Rhodopsin pattern reminiscent of *Drosophila* R7 cells has recently been described for another hemipteran bug, the green rice leafhopper *Nephotettix cincticeps* [107]. Taken together, compared with stochastic or localized patterns, it remains unknown how the ventral eye of insects is patterned to form bands and stripes of discrete, alternating zones with different morphology and Rhodopsin expression.

## Concluding remarks

Here, we have discussed the similarities between insect retinal mosaics and focused on potentially homologous groups of PRs as previously proposed [10]. The ratio of ommatidial subtypes is stable among higher Diptera [33,108,109], yet subtle differences among drosophilids have been reported [110,111]. The data from more distantly related insects reviewed here show that some stochastic mosaics look surprisingly similar (honeybee workers, butterflies, and locusts), raising the possibility that patterning via factors similar to Spineless could be conserved. However, some species may lack any stochasticity and display regional separation of ommatidial subtypes instead (crickets, mosquitoes, and possibly honeybee drones). What could be the advantage of having stochastic patterns over regionalized ones? In the *Drosophila* ventral eye, detectors for color vision (PRs with twisted rhabdomeres) and polarization vision (PRs exhibiting reduced rhabdomeric twist) are distributed stochastically within the same visual field [26,46]. Stochastic distribution could guarantee a bias-free distribution of different detectors, thereby avoiding the loss of sensitivity for one certain stimulus over a larger retinal field, as well as allowing efficient processing between ommatidia. In the rest of the retina, pale, yellow, and DTy ommatidia might have similar effects on the color

discrimination abilities of the animal. Interestingly, all species discussed here manifest specialized ommatidia in the dorsal periphery, suggesting that such features are evolutionary conserved and did not evolve independently (reviewed in [39]). To better understand the evolutionary relation between dorsal or ventral retinal structures, the expression of patterning genes (*IroC*, *Wg*, and *Slp*) and transcription factors expressed in distinct PRs must be compared between species. This is a time-consuming task, but one that has already provided important insight, for instance into how conserved factors govern the dorsoventral patterning of the retina in different insect species [112–114]. An exciting possibility lies in the newest molecular tool for genome editing: CRISPR-Cas9 technology will allow the experimental redesign of various insect retinal mosaics to test the role of certain genes or pathways in any given species [115]. Similar techniques are already being used in insects such as moths and crickets [116–119]. In the future, they will add important facets to the comparative study of retinal mosaics by addressing novel patterning strategies of general interest, such as the definition of retinal stripes.

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