Cellular Innovations and Diversity in the Lepidopteran Compound Eye

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Abstract

Lepidoptera, having co-diversified with flowering plants and adapted to various diel niches, present a remarkable system for studying compound eye cell type diversity. Here we synthesize the latest research regarding lepidopteran eye evolution across different timescales, from species-level variation to family-level changes, and mechanistic levels, from broad anatomical variation to molecular mechanisms responsible for spectral tuning. Opsin duplication, differential expression, and co-expression, combined with lateral filtering pigments, generate diverse spectral sensitivities in photoreceptors. Lateral filtering is particularly important for the convergent evolution of red vision. These diverse photoreceptors combine to form a handful of ommatidial types distributed differentially across eye regions, potentially specializing for distinct behavioral tasks. The coordinated development of these complex retinal mosaics requires precise regulatory mechanisms that we are only beginning to understand. Notably, only a subset of these ommatidial types contribute to color vision, highlighting the need for more research on their roles in motion and spatial vision. We also review support cells providing essential functions such as light insulation or reflection. Future research should focus on identifying ecological pressures driving visual system evolution, genetic bases of diverse retinal mosaics, and neural integration of visual information in Lepidoptera.

Keywords: Butterfly, Color vision, Filter pigment, Moth, Ommatidia, Opsin, Photoreceptor, Spectral sensitivity.

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1. Introduction

Typical of crustaceans and hexapods (including insects), the compound eye is one of two principal visual systems in the animal kingdom, alongside the single-lens camera-type eyes found in vertebrates and cephalopods (Harzsch and Hafner 2006). Butterflies and moths (Lepidoptera) represent one of four major insect superradiations, with most lineages diversifying rapidly with the rise of flowering plants (angiosperms) in the Cretaceous (Mitter et al. 2017; Kawahara et al. 2019). This close association with angiosperms, as herbivores during the larval stage and pollinators as adults, has likely driven the remarkable diversification of the lepidopteran visual system in order to identify suitable host plants and detect flowers (Ehrlich and Raven 1964; Kawahara et al. 2023). Additionally, Lepidoptera exhibit a wide range of diel activity patterns (day vs. night), with more than 40 independent transitions to diurnality, further driving the diversification of the Lepidoptera compound eye (Kawahara et al. 2018).

Numerous comprehensive reviews have explored insect color vision and the diversity of retinal mosaics (Briscoe and Chittka 2001; Stavenga and Arikawa 2006; Wernet et al. 2015; Arikawa 2017; Song and Lee 2018; Schnaitmann et al. 2020; van der Kooi et al. 2021; McCulloch et al. 2022a). However, recent developments have created new opportunities to expand on this foundation. The availability of high-quality Lepidoptera genomes has greatly enhanced our ability to investigate the genetic basis of visual diversity (Mulhair et al. 2023; Wright et al. 2024). Furthermore, an increasing number of studies have linked compound eye structure and function to butterfly behavior, ecology, and evolution (Wainwright et al. 2023; Rossi et al. 2024; Wright et al. 2024; Dang et al. 2025; VanKuren et al. 2025). Together, these advances underscore the need for a comprehensive, up-to-date review of the lepidopteran visual system. In this review, we summarize both shared patterns and clade-specific features of compound eye cell types in the Lepidoptera.

2. Basic structure of the Lepidoptera compound eye

The compound eye consists of many repeated individual units called ommatidia. Most butterflies (superfamily Papilionoidea) studied thus far have the ancestral afocal apposition eye (Fig. 1B), where the lens in each ommatidium forms a small, inverted image (Land and Nilsson 2012). Another major compound eye type, the refracting superposition eye (Fig. 1A), is found in diurnal Hesperiidae (Orridge et al. 1972), nocturnal Hedylidae (Yack et al. 2007), and many moth families (Pirih et al. 2018). Unlike apposition eyes, superposition eyes form a single erected image deeper in the eye by combining light from many lenses (Land and Nilsson 2012). An intermediate eye type is also found in miniature moths (Fischer et al. 2014). Each ommatidium contains photoreceptors as well as support cells such as pigment and cone cells. Photoreceptors are sensory neurons that detect light and convert it into electrical signals. They can be classified in various ways: developmentally, by their position within the ommatidia; molecularly, by the genes they express, especially the light-sensitive opsins; and functionally, by their spectral sensitivity. Broadly, photoreceptors are categorized into inner photoreceptors, which primarily mediate color vision, and outer photoreceptors, which contribute to motion detection (Cook and Desplan 2001).

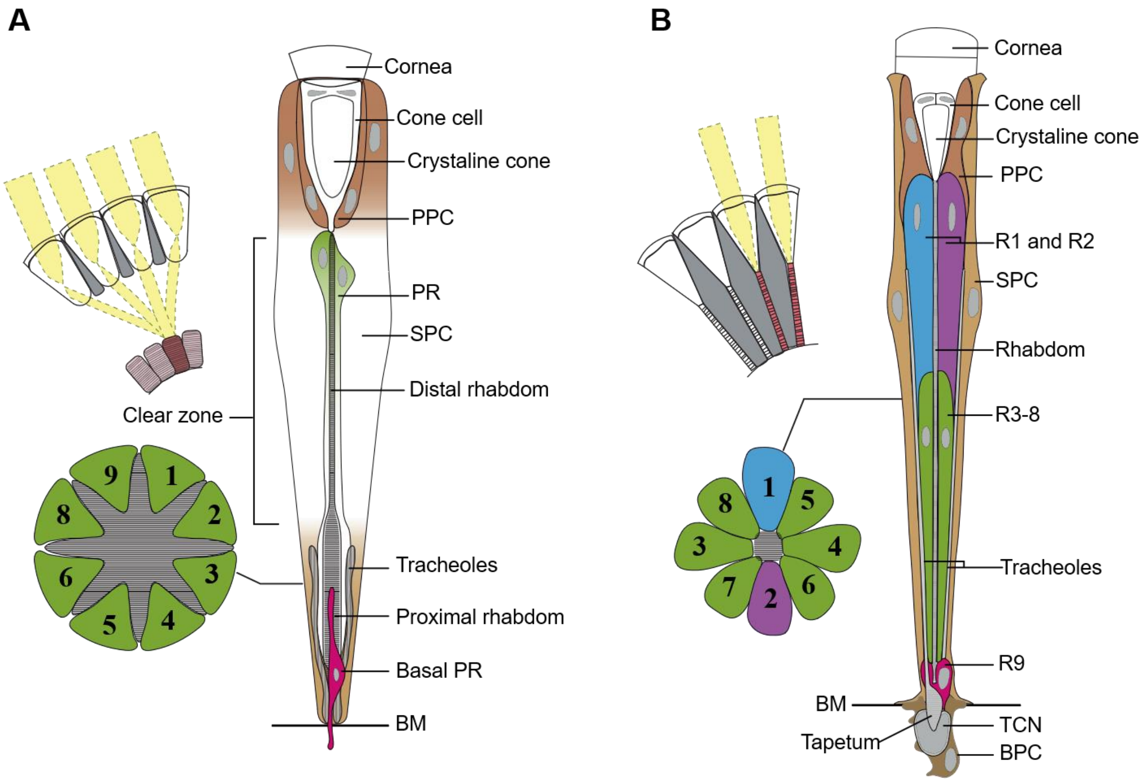

Fig. 1. Anatomy and light paths of superposition and apposition compound eyes.

(A) *Right side*: Anatomy of a superposition eye in the nocturnal corn borer moth (*Ostrinia nubilalis*), adapted from (Belušič et al. 2017). The dioptric apparatus (cornea and crystalline cone) in the distal region is separated from the proximal rhabdom by a clear zone. The nuclei of the basal PRs lie directly beneath the rhabdom. *Top left side*: Light path in a superposition eye, where light from multiple ommatidia passes through the clear zone and converges on a single proximal rhabdom, enhancing light sensitivity at the expense of acuity. *Lower left side*: Cross section at the proximal rhabdom, revealing a characteristic flower-shaped structure formed by microvilli from photoreceptors oriented in different directions. **(B)** *Right side*: Anatomy of an apposition eye in the diurnal small tortoiseshell butterfly (*Aglais urticae*), adapted from (Kolb 1985). The bilobed basal PR (R9) has its nucleus positioned adjacent to the rhabdom. *Top left side*: Light path in an apposition eye, where each ommatidium is optically isolated by heavily pigmented SPCs; only light entering at specific angles reaches the rhabdom. *Lower left side*: Cross section of the incompletely-tiered rhabdom. Abbreviations: PR, photoreceptor; BM, basement membrane; PPC, primary pigment cell; SPC, secondary pigment cell; BPC, basal pigment cell.

In contrast to the well-studied Diptera (flies and mosquitos) ommatidium, which contains one R7 and one R8 inner photoreceptor, the butterfly ommatidium includes an additional inner photoreceptor (two R7 and one R8). Among the major winged insect (Pterygota) orders, this configuration of two R7 cells has only been observed in Lepidoptera and Hymenoptera, two groups that have been studied extensively in the context of color vision (van der Kooi et al. 2021; Gao et al. 2025). The nocturnal moth-butterfly (Hedylidae) represents a notable outlier within the butterfly superfamily, possessing only eight photoreceptors per ommatidium. In contrast, outside the butterfly superfamily, the number of photoreceptors within ommatidia is more variable (Fig. 2). For example, the hawkmoth *Manduca sexta* has fly-like ommatidia (one R7 cell) in the dorsal region and butterfly-like ommatidia (two R7 cells) in the ventral region (White et al. 2003; Gao et al. 2025). In moth species with superposition eyes, each ommatidium can contain 8-16 photoreceptors (Horridge et al. 1977; Belušič et al. 2017; Yang et al. 2024). In keeping with the butterfly color vision literature (Ribi 1987), we refer to the two duplicated fly R7 homologs as R1/2, the fly R8 homolog as R9, and the outer photoreceptors (fly R1-6) as R3-8.

A key structure within the ommatidium is the rhabdom, where visual pigments are concentrated and react to light passing through the lens. All photoreceptors in a lepidopteran ommatidium contribute to the rhabdom by tightly joining their microvilli, forming a fused rhabdom. The combination of fused rhabdom and apposition eye is thought to represent the arthropod ancestral state (Osorio 2007).

The spatial arrangement of the rhabdom is quite variable (Fig. 2). For instance, in Papilionidae and Pieridae, the rhabdom is fully tiered: R1-4 cells contribute microvilli to the distal tier of the rhabdom, while the proximal tier consists of R5-8 microvilli. At the most basal position, R9 contributes to a small section of the rhabdom (Ribi 1978; Arikawa and Uchiyama 1996). In contrast, species in the family Nymphalidae generally have incompletely tiered rhabdoms, where R3-8 contribute their microvilli along the entire length of the rhabdom (Gordon 1977). Exceptions to these patterns occur in some butterfly and moth species. For example, the giant butterfly-moth (*Paysandisia archon*) has two types of ommatidia. In type I, the distal rhabdom consists exclusively of R1/2, and this configuration is also found in the butterfly *Parnassius glacialis* (Matsushita et al. 2012). In type II, the distal rhabdom is split into two sub-rhabdoms, one formed by R2, R3, R5, R6 and the other by R1, R4, R7, R8 (Pirih et al. 2018).

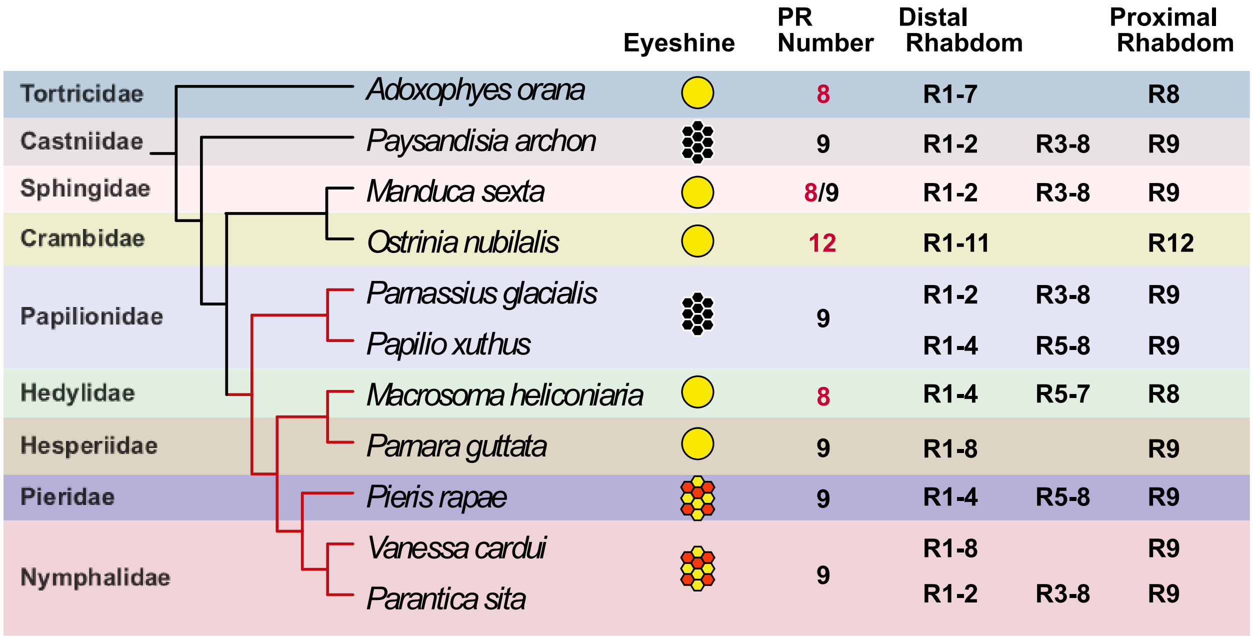

Fig. 2. Evolution of ommatidial anatomical structures in Lepidoptera.

A phylogeny of representative species from various Lepidoptera families is shown, with butterflies (Superfamily *Papilionoidea*) highlighted in red branches. The family-level phylogeny is based on (Kawahara et al. 2019). In the eyeshine column: colorful hexagons, apposition eyes with tapetum; black hexagons, apposition eyes without tapetum; yellow circles, superposition eyes with tapetum. For each species, the total number of photoreceptors per ommatidium is indicated, along with their grouping based on their contribution to the rhabdom, which is organized into two or three tiers. Across all species, regardless of eye type (apposition or superposition), the ommatidium consistently contains a distinct basal photoreceptor. References: *Adoxophyes* (Satoh et al. 2017); *Paysandisia* (Pirih et al. 2018); *Manduca* (White et al. 2003; Gao et al. 2025); *Ostrinia* (Belušič et al. 2017); *Parnassius* (Matsushita et al. 2012); *Papilio* (Arikawa and Uchiyama 1996); *Macrosoma* (Yack et al. 2007); *Parnara* (Shimohigashi and Tominaga 1986); *Pieris* (Ribi 1978); *Vanessa* (Briscoe et al. 2003); *Parantica* (Nagloo et al. 2020).

3. Evolution of lepidopteran opsin genes

The spectral sensitivity of photoreceptors is primarily determined by the visual pigments they express. In arthropods, these visual pigments are composed of rhabdomeric-type opsin (r-opsin) proteins, members of the G protein-coupled receptor family, that covalently bind to the retinal-based chromophores and respond to different wavelengths of light (Henze and Oakley 2015). Ancestrally, Lepidoptera possess three types of r-opsins with distinct peak sensitivity: green-sensitive long-wavelength (LW) opsins, blue-sensitive short-wavelength (B) opsins, and ultraviolet-sensitive (UV) opsins (Briscoe and Chittka 2001; Stavenga and Arikawa 2006; Briscoe 2008). Most photoreceptors follow the 'One Receptor' rule of sensory neurons, expressing a single opsin gene per cell (Mazzoni et al. 2004). However, numerous instances of opsin co-expression have been observed in butterfly photoreceptors (Fig. 3).

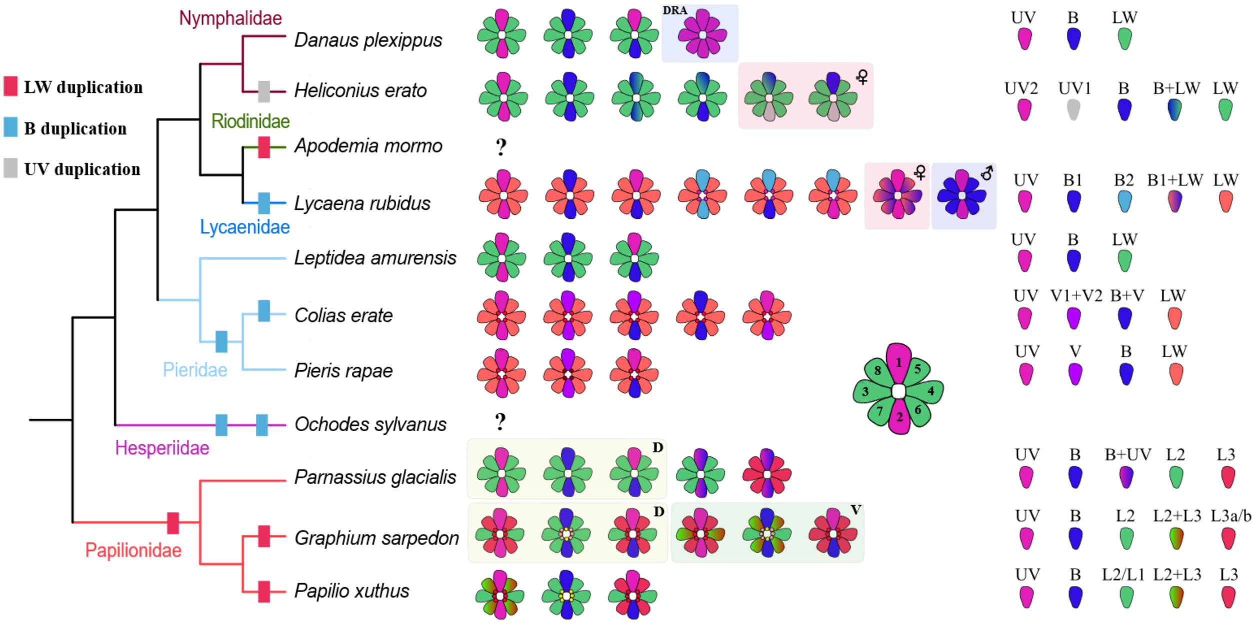

Fig. 3. Evolution of retinal mosaics in butterflies.

Left panel: the phylogeny of several butterfly species with well-characterized opsin expression patterns, with gene duplication events marked along the branches. The phylogeny is based on (Kawahara et al. 2023). *Middle panel:* ommatidial types for each species based on opsin expression. The enlarged ommatidium indicates the position of R1–R8 cells (R9 not shown). Types that are sex-specific or region-specific are highlighted with boxes (D: dorsal, V: ventral, DRA: dorsal rim area). The presence of perirhabdomal filtering pigments is indicated for *Colias*, *Pieris*, *Graphium*, and *Papilio*. *Right panel:* the diversity of photoreceptor types that compose the retinal mosaics. Co-expression is indicated by mixed colors within a cell and plus signs. References: *Danaus* (Sauman et al. 2005); *Heliconius* (McCulloch et al. 2017); *Apodemia* (Frentiu et al. 2007); *Lycaena* (Sison-Mangus et al. 2006); *Leptidea* (Uchiyama et al. 2013); *Colias* (Ogawa et al. 2012); *Pieris* (Arikawa et al. 2005); *Ochodes* (Mulhair et al. 2023); *Parnassius* (Awata et al. 2010); *Graphium* (Chen et al. 2016); *Papilio* (Arikawa 2003).

Outer photoreceptors (R3-8) mainly express LW opsins. The inner photoreceptor R9 has also been shown to express LW opsins in species such as *Papilio glaucus* and *Vanessa cardui* (Briscoe et al. 2003; Briscoe 2008). However, due to its small size and basal position within the ommatidium, the opsin expression of R9 remains poorly understood in most species. Inner photoreceptors R1 and R2 typically express UV or B opsins. Stochastic expression of UV or B opsins in R1/2 results in three stochastically distributed ommatidial types: UV-UV, UV-B, and B-B (Perry et al. 2016). This type of retinal mosaic is typical of most butterflies and moths (White et al. 2003; Arikawa 2003) and is also found in honeybees (Wakakuwa et al. 2005).

One key mechanism for expanding the spectral diversity of photoreceptors is gene duplication and divergence. Duplicated opsins can evolve distinct peak sensitivities by changing amino acids in the chromophore-binding pocket, also known as spectral tuning. These opsin paralogs can acquire novel expression patterns in new cell types or specialize among subsets of the original cell type (Briscoe 2008). Gene duplications of opsins in Lepidoptera have been documented since the early-day cDNA cloning and *in situ* hybridization studies (Kitamoto et al. 1998; Briscoe 2000). Following the publication of the first moth genome (*Bombyx mori*; Xia et al. 2004) and the first butterfly genome (*Danaus plexippus*; Zhan et al. 2011), an increasing number of lepidopteran genomes and transcriptomes have been sequenced using next-generation sequencing technologies. These datasets have enabled broader taxonomic surveys of opsin gene diversity (Sondhi et al. 2021; Kuwalekar et al. 2022). However, opsin gene copy number may be underestimated in fragmented genome assemblies. This limitation is now being addressed with chromosome-level genome assemblies produced using third-generation sequencing methods, such as those generated by the Darwin Tree of Life project (Mulhair et al. 2023). Opsin gene duplications are now recognized as more widespread across Lepidoptera than previously thought (Table S1).

3.1. Long-wavelength opsin duplication and expression

LW opsin duplications are widespread across Lepidoptera (Sondhi et al. 2021; Kuwalekar et al. 2022; Mulhair et al. 2023). Within the butterfly superfamily, LW opsin duplications have been identified in Papilionidae, Riodinidae, Nymphalidae, and Hesperiidae (Fig. 3). They are also common across multiple moth families. One of the most ancient opsin duplication events in Lepidoptera is the duplication of LW opsin in the Noctuoidea superfamily, which occurred approximately 80 million years ago. All current Noctuoidea species share an intronless *LWS2* gene, likely produced by the retrotransposition of the ancestral *LWS1* copy (Mulhair et al. 2023).

At the base of Papilionidae, an LW opsin duplication event generated two opsins: the ancestrally green-sensitive L2 and the red-sensitive L3. The peak absorption wavelength of L3 is approximately 570 nm in *Papilio xuthus* (Kitamoto et al. 1998; Saito et al. 2019). *Parnassius glacialis* butterflies (subfamily Parnassiinae) only have these two LW opsin copies from the ancestral duplication (Awata et al. 2010). In contrast, *Graphium sarpedon* (subfamily Papilioninae), a butterfly with extreme spectral richness, possesses three LW opsins (L2, L3a, and L3b), due to a duplication of L3 that is shared among the Leptocircini tribe. In *Graphium*, dorsal R3-8 photoreceptors only express one LW opsin per cell (either L2 or L3a), while ventral R3-8 photoreceptors can co-express two or three LW opsins in a single photoreceptor, generating at least five types of long-wavelength-sensitive photoreceptors (Chen et al. 2016). In *Papilio* butterflies, a separate, genus-specific duplication of L3 produced three total LW opsins: L2, L3, and L1. Similar to *Graphium*, each R3-8 photoreceptor can express one or two LW opsins, although the co-expression of all three LW opsins has not been observed in *Papilio* (Kitamoto et al. 1998; Briscoe 2008).

Although LW opsin duplications have been documented in both diurnal and nocturnal Lepidoptera, not all duplicated copies function in color vision or brightness contrast. For example, in *Bombyx mori*, one duplicated LW opsin is expressed in the larval brain tissue, where it regulates photoperiodic responses (Shimizu et al. 2001).

3.2. Blue opsin duplication and expression

The most well-characterized B opsin duplication events have been documented in the butterfly families Lycaenidae and Pieridae (Fig. 3). In Lycaenidae, an ancestral B opsin duplication gave rise to two B opsin copies, B1 and B2, which are shared across the family (Bernard and Remington 1991; Sison-Mangus et al. 2006). These opsins are expressed in R1/2 photoreceptors in distinct, non-overlapping patterns with each other and with UV opsins. As a result, *Lycaena rubidus* exhibits six R1 and R2 subtype combinations: UV-UV, UV-B1, UV-B2, B1-B1, B1-B2, and B2-B2 (Sison-Mangus et al. 2006). In *L. rubidus*, B1 also shows a novel expression pattern in R3-8 photoreceptors, which ancestrally expressed only LW opsins. In females, these photoreceptors in the dorsal eye co-express LW and B1 opsins, while in males, the same cells only express B1. This sexually dimorphic expression pattern has been linked to sexual selection and the prevalence of blue pigments on Lycaenidae wings (Sison-Mangus et al. 2006).

Two blue opsin duplication events have been identified in Pieridae. The first is an ancestral blue opsin duplication that occurred at the base of the Coliadinae and Pierinae lineages, generating a blue-sensitive opsin (B, λ_{max} at 450nm) and a violet-sensitive opsin (V, λ_{max} at 420nm) with a spectral shift toward the UV range (Wakakuwa et al. 2010). In Coliadinae, the V opsin underwent a second duplication (Arikawa et al. 2005; Awata et al. 2009). As a result, Coliadinae species possess three blue opsins (B, V1, V2), while Pierinae species have two (B and V). Surprisingly, the duplication of blue opsins does not increase the total number of ommatidial types in *Pieris rapae*; only three ommatidial types (UV-UV, UV-B, and V-V) are observed. Notably, V opsins are expressed only in ommatidia that lack the ancestral UV or B opsin expression (Arikawa et al. 2005).

In *Colias erate*, the violet opsins V1 and V2 are always co-expressed in R1/2 photoreceptors. Additionally, a novel photoreceptor subtype has been identified in *Colias* that expresses all three blue opsins (B, V1, and V2), representing the highest number of co-expressed opsin genes within a single photoreceptor (Ogawa et al. 2012). Beyond the well-characterized blue opsin duplications in Pieridae and Lycaenidae, similar duplications have also been reported in several Hesperiidae butterflies and even in an Erebidae moth species (Mulhair et al. 2023).

3.3. Ultraviolet opsin duplication and expression

Unlike LW and B opsin duplications, UV opsin duplications are rare in Lepidoptera. The only confirmed UV opsin duplication event occurred in the common ancestor of all *Heliconius* butterflies, generating UV1 and UV2 (Briscoe et al. 2010). In the *erato*/*sara*/*sapho* clade, the ancestral *UVRh2* is located on an autosome but the duplicated *UVRh1* is located on the female-specific W chromosome, resulting in sexually dimorphic UV opsin expression (Chakraborty et al. 2023). In females of this clade, two distinct UV photoreceptor cell types have been identified, each expressing either UV1 or UV2 (McCulloch et al. 2016, 2017). Behavioral studies further support this sexual dimorphism, showing that female *H. erato* and *H. charithonia* possess true UV color vision (Finkbeiner and Briscoe 2021; Chakraborty et al. 2023).

In the other major *Heliconius* clade (*melpomene*/*doris*), both *UVRh1* and *UVRh2* are located on autosomes. Since the sister group of this *melpomene*/*doris* clade, *H. aoede*, only expresses *UVRh2* in males (based on RNA-seq data), the most parsimonious explanation is that *UVRh1* was initially duplicated onto the W chromosome and later translocated to an autosome in the *melpomene*/*doris* lineage (McCulloch et al. 2017). Within this clade, female *H. doris* have an additional UV photoreceptor cell type that co-expresses UV1 and UV2 almost equally, while *H. ethilla* in the silvaniform lineage lost UV2 expression entirely due to the pseudogenization (McCulloch et al. 2017). Even within a single *H. cydno* species complex, peak sensitivities of UV photoreceptors vary significantly across subspecies and sexes, which are driven by shifts in the relative expression level of UV1 and UV2 (Buerkle et al. 2022; VanKuren et al. 2025).

Overall, a single genus-specific UV opsin duplication event, followed by chromosomal translocation and lineage-specific pseudogenization, has resulted in at least eight distinct R1/2 ommatidial types (McCulloch et al. 2017). This complex pattern of gene expression evolution highlights that understanding spectral diversity requires not only broad taxonomic sampling, but also dense sampling within genera, as closely-related species can exhibit substantial differences.

3.4. Co-expression of multiple opsins

As noted previously, photoreceptors broaden their spectral sensitivity by co-expressing multiple opsin genes within the same cell (Arikawa et al. 2003). For example, *Colias* butterflies co-express V1 and V2 opsins, derived from a duplication at the base of the Coliadinae subfamily (Ogawa et al. 2012). Similar co-expression of opsins originating from genus- or family-level duplications is also observed in other species (Arikawa et al. 2003; Briscoe et al. 2010; Chen et al. 2016).

In contrast, the co-expression of opsins from different spectral classes (UV, B, LW) is much rarer in Lepidoptera. In *Parnassius glacialis*, a subset of ventral R1/2 photoreceptors co-express UV and B opsins (Awata et al. 2010), similar to the ventral stripe R7 photoreceptors of the mosquito *Aedes aegypti* (Hu et al. 2011). Even more surprising is the co-expression of B and LW opsins, which are typically restricted to inner and outer photoreceptors, respectively. In *Lycaena rubidus*, female R3-8 photoreceptors co-express B1 and LW opsins (Sison-Mangus et al. 2006). Across the Heliconiini clade (including *Heliconius*, *Eueides*, and *Dryas*), multiple retinal mosaics feature R1/2 photoreceptors that co-express B and LW opsins (McCulloch et al. 2017). These broad-spectrum photoreceptors generate three additional ommatidial types (McCulloch et al. 2017; Chakraborty et al. 2023). Together, these examples illustrate the remarkable flexibility of opsin expression in Lepidoptera, particularly the unexpected expression of outer photoreceptor opsins in inner photoreceptors, and vice versa.

3.5. Temporal expression pattern

Fully differentiated ommatidia with all nine photoreceptors and four cone cells can be found in the pupal retina of butterflies as early as day 1, representing approximately 10% of pupal development (Arikawa et al. 2017; Gao et al. 2025). However, the rhabdom is not completed until the end of pupal development or shortly after adult eclosion. In *Papilio xuthus*, the onset of opsin gene expression occurs during pupal development and follows a consistent temporal sequence: UV and B opsins are expressed first, followed by L2, then L3, and finally L1 (Arikawa et al. 2017). The ancestral green-sensitive L2 opsin initially appears in all R3-8 photoreceptors. In a subset of ommatidia, L2 is later replaced by the red-sensitive L3 in R5-8 photoreceptors. The genus-specific L1 opsin is only detectable after day 9 and is restricted to R3/4 photoreceptors, which continue to co-express L2 (Arikawa et al. 2017). Interestingly, the temporal order of opsin expression in *P. xuthus* mirrors the evolutionary sequence in which these opsins arose, suggesting a case of "ontogeny recapitulating phylogeny"(Domazet-Lošo and Tautz 2010; Kalinka et al. 2010). Whether this pattern holds true for other duplicated opsins remains unknown. More comparative studies on the temporal expression pattern of duplicated opsin genes need to be done, especially in species with multiple opsin duplications, such as the *Colias* butterflies with their three middle-wavelength opsins.

4. Lateral filtering and convergent evolution of red photoreceptors

The evolution of red color vision may serve multiple functions, including mate recognition, flower detection, and host plant discrimination for oviposition (Fig. 4). While red-sensitive photoreceptors are rare in Hymenoptera, they have evolved repeatedly and are widespread in Lepidoptera (Briscoe and Chittka 2001). The evolution of red-sensitive photoreceptors both expands the visual range and enhances wavelength discrimination in the long-wavelength spectrum.

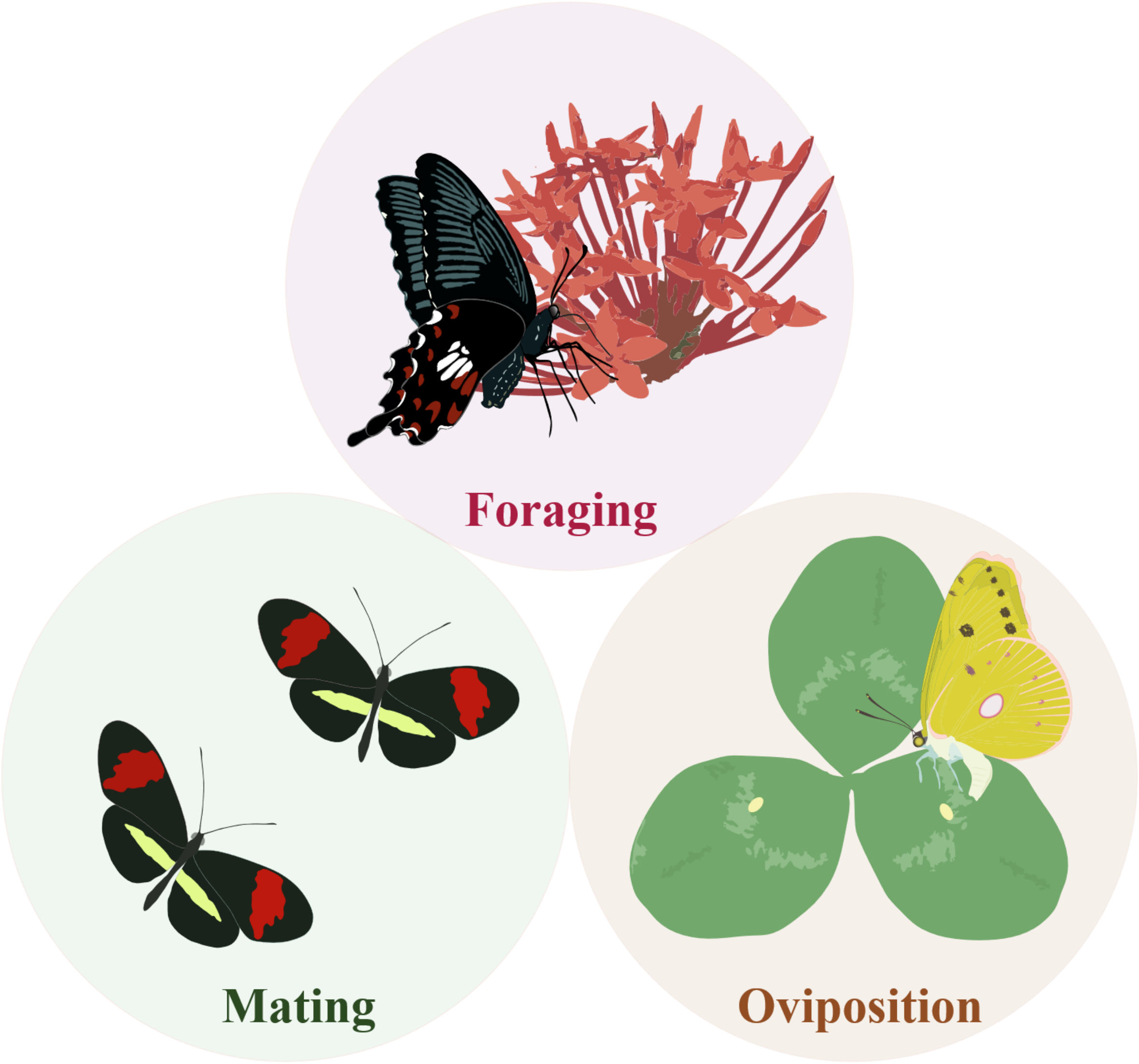

Fig. 4. Behavioral ecology of red color vision.

Top: *Papilio polytes* (family Papilionidae) feeding on red *Ixora* flowers. *Lower left*: A male *Heliconius melpomene* (family Nymphalidae) chasing a conspecific female with bright red patches on the forewings. *Lower right*: *Colias erate* (family Pieridae) laying eggs on *Trifolium* leaves.

Sensitivity to long wavelengths, including red light, allows butterflies to exploit nectar-rich red flowers, which are typically pollinated by birds and avoided by bees (Johnson and Bond 1994; Chen et al. 2020b). Butterflies in the family Papilionidae, Pieridae, and Nymphalidae are common visitors to these butterfly-pollinated flowers (Hirota et al. 2013; Kiepiel and Johnson 2014). In addition to flower discrimination, the evolution of red-sensitive receptors, in addition to the ancestral green receptors, may aid butterflies in selecting young versus mature leaves for oviposition (Kelber 1999). In nocturnal moths, red color vision may play a different role, potentially facilitating the detection of food or host plants under dim, red-shifted nocturnal light conditions (Warrant and Somanathan 2022; Tang et al. 2024).

The presence of red photoreceptors can be detected in several ways: behaviorally through color discrimination tests, functionally by electrophysiology, or indirectly by the identification of red filter pigments via histology or eyeshine. Many diurnal butterflies with apposition eyes, with the exception of Papilionidae species, exhibit colorful eyeshine caused by a reflecting tapetum located at the proximal end of each ommatidium (Miller and Bernard 1968). Although red sensitivity has evolved multiple times independently in Lepidoptera, the selective pressures driving this convergence remain poorly understood (Briscoe and Chittka 2001).

4.1. Filter pigments in Lepidoptera compound eyes

The spectral sensitivity of photoreceptors is determined not only by the photosensitive opsins they express but also by the presence of photostable filter pigments within the ommatidia. These filter pigments are stored in membrane-bound compartments known as pigment granules, which are lysosome-related organelles (Dell'Angelica et al. 2000). Pigment granules are found in both pigment cells and photoreceptors, most of which exhibit relatively uniform absorption across the 300–700 nm wavelength range (Höglund et al. 1970). In both butterflies and moths, the radial or vertical movement of these pigment granules within an ommatidia can function like a pupil, regulating light input to the rhabdom during light adaptation (Stavenga and Kuiper 1977; Satoh et al. 2017). In addition, these pigments can absorb stray light from adjacent ommatidia, ensuring that each ommatidium primarily receives coaxial light. This function enhances visual acuity in species with apposition-type eyes (Linzen 1974). Beyond functioning as pupil filters, some pigment granules exhibit maximal absorption at specific wavelength ranges, thereby serving as spectral filters (Stavenga 1995). Among these, red filter pigments, characterized by strong absorption of wavelengths shorter than 600 nm, were first identified in the butterfly species *Pieris rapae* (Ribi 1978). Unlike other pupillary pigments in photoreceptors or pigment cells, these red pigment granules do not move substantially in response to light, and are concentrated in clusters in the photoreceptor soma, near the rhabdom. They absorb short-wavelength light as light pass through the rhabdom, a process known as lateral filtering (Ribi 1978). As a result, the presence of red filter pigments shifts the peak sensitivity of photoreceptors toward longer wavelengths and narrows the sensitivity spectrum, effectively creating distinct long-wavelength photoreceptors. This enables color opponency and finer discrimination across the green-to-red spectrum (Fig. 5).

4.2. The evolution of red photoreceptors in Papilionidae

Papilionidae represents a special case in the evolution of red photoreceptors, characterized by both LW opsin duplications and the presence of red filter pigments. The duplication of LW opsins enables a broader range of peak spectral sensitivities (Frentiu et al. 2007). Behavioral experiments show that *Papilio xuthus* can discriminate wavelength differences as small as 10 nm, even in the red range around 620 nm (Koshitaka et al. 2008).

Four types of filter pigments have been identified in the three ommatidial types of *P. xuthus* (tribe Papilionini). Each ommatidial type shows a coordinated combination of R1/2 opsin expression and filter pigments in R1–8. Based on R1/2 opsin expression, the three types are: type I (UV-B), type II (UV-UV), and type III (B-B) (Kitamoto et al. 1998). In the distal region, purple pupillary pigment granules are found in R1/2 cells across all ommatidia. The R3-8 cells of each ommatidium contain clusters of pigment granules, either red (type I and type II) or yellow (type III), located within 1 μm of the rhabdomere boundary. Additionally, type II ommatidia possess UV-absorbing fluorescent pigments, specifically 3-hydroxyretinols. These UV-absorbing pigments modify the spectral sensitivities of UV receptors (R1/2) and double-peak green receptors (R3/4) in type II ommatidia, converting them into narrow-band violet receptors and single-peak green receptors, respectively (Arikawa and Stavenga 1997; Arikawa 2003). In *Drosophila*, 3-hydroxyretinols are hypothesized to function as sensitizing pigments, transferring excitation energy to rhodopsins (Vogt and Kirschfeld 1983).

In *P. xuthus*, red-sensitive proximal photoreceptors exhibit a narrow peak at 600 nm, resulting from L3 opsin expression (λ_{max} at 575 nm) combined with red perirhabdomal filter pigments (Arikawa et al. 1999). Histology studies show that L3 is exclusively expressed in the proximal R5-8 cells of red ommatidia. (Arikawa 2003). This tight association between red filter pigments and the red-sensitive L3 is also suggested in the distantly-related *Parnassius glacialis* (tribe Parnassiini), where a subset of the ventral ommatidia contain red pigments and express L3 in R3-8 (Awata et al. 2010). In another species, *Troides aeacus formosanus* of the tribe Troidini, a sister tribe to Papilionini, two red receptors (λ_{max} at 610 nm and 630 nm) are found in ommatidia with pale-red and deep-red pigments, respectively (Chen et al. 2013; Condamine et al. 2018). The most striking example of the red receptor diversity is found in *Graphium sarpedon*, a species of the tribe Leptocircini. Electrophysiological recordings reveal five distinct subclasses of red receptors, including a deep-red receptor peaking at 640 nm, which has been histologically identified as the L3a-expressing proximal photoreceptor (Chen et al. 2016).

4.3. The evolution of red photoreceptors in Pieridae

Despite having a single copy of the LW opsin gene, Pieridae butterflies possess some of the most diverse red photoreceptors among Lepidoptera. Similar to *Papilio* butterflies, the rhabdom of Pieridae is fully tiered. In *Colias* butterflies, the rhabdom in ventral ommatidia is divided into proximal and distal tiers by a strong constriction, enhancing the filtering effect of the red perirhabdomal pigments in R5-8 (Arikawa et al. 2009). The most red-shifted green photoreceptor ever recorded in insects is found in *Colias erate*, with a peak sensitivity at 660 nm (Pirih et al. 2010). By varying the spatial distribution of red perirhabdomal pigments and introducing a female-specific orange perirhabdomal pigment, female *C. erate* possess three red photoreceptor types with peak sensitivity at 610 nm, 650 nm, and 660 nm (Ogawa et al. 2013). This expansion pushes their color discrimination range close to the far-red limit of approximately 700 nm. In contrast, male *C. erate* butterflies have only one type of red receptor with peak sensitivity at 660 nm. In the dorsal eye region, which is not sexually dimorphic, R5-8 in both sexes are maximally sensitive at 600–620 nm, due to a moderate filtering effect from lower filter pigment density and weak constriction (Ogawa et al. 2013). Unlike *C. erate*, both male and female *Pieris rapae* butterflies have three red photoreceptor types in their ventral ommatidia, with peak sensitivities at 610 nm, 630 nm, and 640 nm. These spectral differences arise from the distinct red pigment granules present in each of the three ommatidial types, likely due to varying pigment densities within the granules (Blake et al. 2019).

If all photoreceptors contributed equally to color vision, Pieridae butterflies would be expected to have strong color discrimination in the red range. However, field observations show that neither *Colias* nor *Pieris* butterflies exhibit a preference for red flowers. In a feeding-based behavioral experiment, *P. rapae* butterflies trained on red paper disks preferentially visited orange and purple disks over red, suggesting either poor discrimination within the orange-red spectrum or that red color vision is primarily utilized in non-feeding contexts, such as oviposition (Arikawa et al. 2021).

In addition to red perirhabdomal pigments, *P. rapae* males have a fluorescent pigment in type II ommatidia that emits fluorescence under 420 nm excitation. This pigment turns the violet-sensitive R1/2 photoreceptor into double-peak blue receptors (Qiu et al. 2002; Arikawa et al. 2005). A similar filtering effect occurs in *C. erate* male type I ommatidia and female type II ommatidia (Ogawa et al. 2012).

Anthocharis butterflies (subfamily Pierinae) represent a secondary loss of the ommatidial heterogeneity in Pieridae. Only two ommatidial types are distinguishable, based on the arrangement of red perirhabdomal pigments in R5-8. In round-type ommatidia, red pigments are located in the distal half of the ommatidium, whereas in trapezoidal-type ommatidia, they are confined to the proximal third (Takemura et al. 2007).

4.4. The evolution of red photoreceptors in Lycaenidae

Lycaenidae butterflies achieve long-wavelength color vision through a combination of spectral tuning of their B and LW opsins and lateral filtering. The rhabdom structure of Lycaenidae is not fully tiered, based on the electron microscopy study in *Eumaeus atala* (Liénard et al. 2021). R1 and R2 only contribute their microvilli to the distal portion of the rhabdom, while R3-8 contribute the majority of microvilli throughout the rhabdom (Liénard et al. 2021). In *Lycaena rubidus*, a pink filter pigment is found exclusively in the R5-8 of the ventral eye ommatidia that express B2, a green-shifted B opsin (Sison-Mangus et al. 2006). Across Lycaenidae, many species have also evolved red-shifted LW opsins with peak sensitivities between 564 nm and 571 nm, compared to the ancestral peak near 540 nm (Frentiu et al. 2007; Liénard et al. 2021). In

Polyommatus icarus, this coordinated shift in B and LW opsins, likely enables them to discriminate color in the green wavelength range, up to 560 nm. However, behavioral experiments show that *P. icarus* cannot differentiate colors in the red range (570–640 nm), indicating that their long-wavelength color vision does not extend into the true red spectrum (Sison-Mangus et al. 2008). One possible explanation is the absence of pink filter pigments in the distal ommatidia, which reduces spectral filtering for LW photoreceptors and consequently limits their sensitivity in the red spectrum (Sison-Mangus et al. 2006).

4.5. The evolution of red photoreceptors in Nymphalidae

Although most Nymphalidae species possess only one LW opsin and one B opsin, true red color vision has been verified through behavioral experiments in nymphalid species *Heliconius erato* (Zaccardi et al. 2006) and *Danaus plexippus* (Blackiston et al. 2011). In *Heliconius*, two types of filtering pigments have been identified, with peak absorbance at approximately 450 nm and 560 nm. The red pigment (λ_{max} at 560 nm) is likely ommin, a type of sulfur-containing ommochrome commonly found in insect eyes (Höglund et al. 1970). The presence of these red filter pigments is closely associated with the presence of red-sensitive photoreceptors in Nymphalidae.

A novel class of green-sensitive photoreceptors that hyperpolarize in response to red light (Fig. 6) has been identified across multiple Nymphalidae subfamilies (Belušič et al. 2021). These green-positive, red-negative (G+R-) cells have been allocated to the R1/2 positions and are observed exclusively in species with red eyeshine, which indicates the presence of red filter pigments (Belušič et al. 2021). Co-expression of LW and B opsins in R1/2 photoreceptors has been detected throughout the Heliconiini clade using antibody staining (McCulloch et al. 2022b; Chakraborty et al. 2023). These cells likely correspond to the G+R- photoreceptors involved in red-green color opponency. Within this circuit, the red opponent units (R–) are thought to be the basal photoreceptors R9 (Belušič et al. 2021; Ilić et al. 2022; Pirih et al. 2022). While red-sensitive photoreceptors have been directly recorded in multiple *Heliconius* species (McCulloch et al. 2017, 2022b; VanKuren et al. 2025), the precise identity of these red receptors (whether they correspond to the R9 cell or R3–8 cells) remains unconfirmed.

This R9 localization of red receptors represents a striking contrast to the R3-8 red receptors found in Papilionidae and Pieridae (Fig. 5). The rhabdom in nymphalids is not fully tiered (Kolb 1985), with R3-8 contributing microvilli throughout much of the rhabdom, potentially making R9 better suited to receive light filtered by red pigments. Despite extensive characterization of R1/2-based ommatidial types in *Heliconius*, the relationship between R1/2 opsin expression and the presence of red filter pigments remains unresolved (Buerkle et al. 2022). One hypothesis based on electrophysiological data is that broadband green R1/2 photoreceptors are restricted to red-reflecting ommatidia, but histology studies are needed to confirm this association.

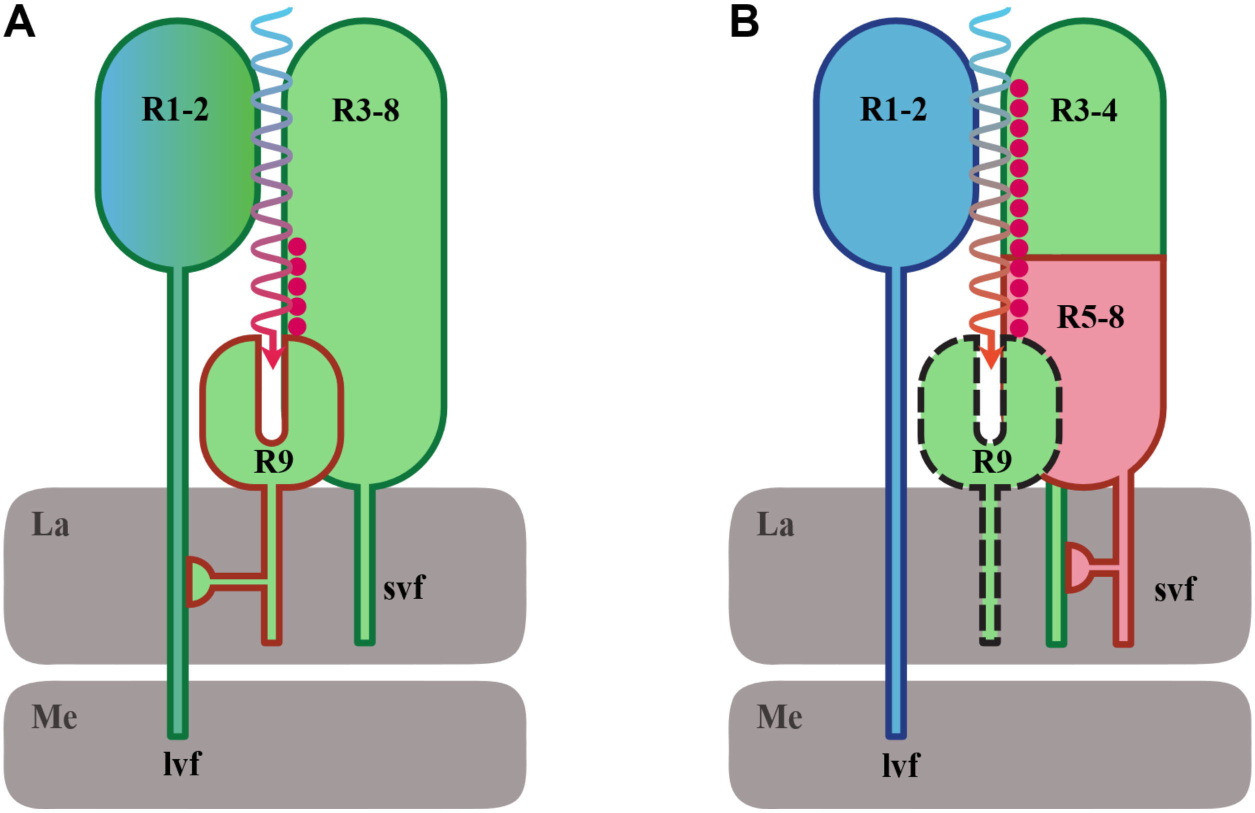

Fig. 5. Convergent evolution of red-green opponency

Schematic representation of red-green opponency mechanisms in Nymphalidae (A) and Papilionidae (B). Photoreceptor outline colors indicate green-, blue-, or red-sensitive cells, while the fill colors represent the opsins they express. **(A)** In Nymphalidae, green-sensitive R1 or R2 photoreceptors (which co-express B and LW opsins) receive direct inhibitory input from red-sensitive R9 photoreceptors (Belušič et al. 2021). The presence of red perirhabdomal pigments shifts the sensitivity of R9 photoreceptors from green to red. **(B)** In Papilionidae, the role of R9 remains unclear (marked with a dashed line). Green-sensitive R3–4 receive inhibitory input from proximal red-sensitive R5–8, driven by a combination of red-sensitive opsin expression and lateral filtering (Chen et al. 2020a). Abbreviations: La, lamina; Me, medulla; lvf, long visual fiber; svf, short visual fiber.

Complex retinal mosaics with red-reflecting ommatidia are found in both sexes of many nymphalids, including early-diverging Danaini (Blackiston et al. 2011). However, the red perirhabdomal pigments have been lost multiple times in Nymphalini (Briscoe and Bernard 2005) and Apaturini (Pirih et al. 2022), which retain ancestral trichromatic color vision, consisting of UV-, blue- and green-sensitive photoreceptors. In Argynnini butterflies, females have secondarily lost the red-reflecting ommatidia, while males retain an expanded retinal mosaic with red-sensitive photoreceptors (Ilić et al. 2022). Overall, the gain and loss of red lateral filtering pigments appear to be highly evolutionarily labile.

5. Regional differences and visual ecology

Dorsal-ventral variation in the compound eye is common across Lepidoptera. In many species, the dorsal region of the eye retains a more conserved and likely ancestral arrangement of ommatidia, characterized by fewer ommatidial types and the absence of fluorescent or perirhabdomal filtering pigments (Qiu and Arikawa 2003; Awata et al. 2010; Ogawa et al. 2013; Chen et al. 2016). The dorsal and ventral regions of the eye can also differ structurally. In *Leptidea amurensis*, the ventral eye exhibits a distinctive rough appearance caused by an irregular distribution of facets in two distinct sizes (Uchiyama et al. 2013). The most extreme example of this is found in the hawkmoth *Manduca sexta*, where the dorsal ommatidia structurally resemble those of ancestral winged insects with only a single R7 cell (White et al. 2003; Gao et al. 2025). These differences between ventral and dorsal eye regions likely reflect their distinct roles in visual ecology. The ventral eye region is thought to be important for behaviors such as host plant recognition and mate detection, while the dorsal eye may be more important for predator detection. However, exceptions exist. In highly territorial *Lycaenae* butterflies, the dorsal region is sexually dimorphic. Males express B1 opsins in R3-8 photoreceptors, which may enhance their ability to detect rival, conspecific males (Sison-Mangus et al. 2006).

In many insects, including Lepidoptera, ommatidia in a small region of the compound eye, known as the dorsal rim area (DRA), are anatomically specialized for detecting polarized skylight (Labhart and Meyer 1999). Although debated, detection of polarized UV light may play an important role in flight orientation in monarch butterflies (*Danaus plexippus*), which are renowned for their long-distance migration (Sauman et al. 2005; Stalleicken et al. 2005). In the monarch butterfly, each DRA ommatidium contains two anatomical types of photoreceptors with mutually orthogonal microvilli, providing the basis for polarization antagonism (Reppert et al. 2004). To avoid interference with color information, R1-8 in monarch DRA ommatidia express UV opsins exclusively (Sauman et al. 2005). A similar expression pattern is observed in the moth *Manduca sexta*, where UV opsin is the only opsin expressed in the DRA (White et al. 2003). Additionally, the monarch DRA lacks functional tapeta, a reflective structure found in other parts of the eye, representing a unique modification of DRA ommatidia in Lepidoptera (Labhart et al. 2009).

Interestingly, highly polarization-sensitive photoreceptors have also been found outside the DRA. In the European corn borer moth (*Ostrinia nubilalis*), distal blue-sensitive photoreceptors in the main retina exhibit strong polarization sensitivity (Belušič et al. 2017). Similar polarization-sensitive ommatidia outside the DRA have also been observed in *Drosophila* where they may play a role in sensing the reflection from water (Wernet et al. 2012).

6. Molecular logic underlying diverse retinal mosaics

Comprehensive reviews on retinal mosaics across insects are available in (Wernet et al. 2015; McCulloch et al. 2022a). Here, we highlight the unique challenges and opportunities in uncovering the molecular logic that shapes the retinal mosaic in butterflies and moths. In *Papilio* *xuthus*, previous studies have shown that two independent stochastic decisions regarding expression of the transcription factor *spineless* in R1/2 photoreceptors give rise to three ommatidial types (Perry et al. 2016). This mechanism is similar to the pale vs. yellow ommatidial fate decision in *Drosophila* (Wernet et al. 2006). Stochastic *spineless* expression not only determines the opsin identity in R1/2 (B or UV) but also coordinates other features of the whole ommatidium, including LW opsin expression in R3-8 and the presence of red perirhabdomal or fluorescent filter pigments (Perry et al. 2016). This tight coordination of filtering pigments and opsins across all photoreceptors within an ommatidium is likely crucial for efficient downstream visual processing, as axons of all nine photoreceptors from the same ommatidium project through the same cartridge in the lamina (Matsushita et al. 2022).

In *Heliconius* and other Nymphalidae butterflies, the presence of red filtering pigments and broadband green-sensitive R1/2 cells results in at least six types of ommatidia. However, the underlying logic generating this expanded retinal mosaic remains unclear. A simple three-way stochastic choice of broadband/UV/B photoreceptors cannot explain the relative proportion of UV-B, B-B, and UV-UV observed. Furthermore, in females of the *Heliconius erato/sara/sapho* clade, this complexity is increased by an additional stochastic choice between UV1 or UV2 in R1/2.

The stochastic expression of *spineless* can be modified regionally to generate dorsal-ventral specialization. In *Drosophila*, for example, the dorsal third of the retina contains yellow R7 cells co-expressing Rh3 and Rh4, which are typically restricted to expressing only Rh4. This co-expression is driven by reduced inhibition from lower *spineless* expression and activation from the Iroquois complex transcription factors (Thanawala et al. 2013). The *Lycaenae* butterflies, with both dorsal-ventral retinal specialization and sexually dimorphic dorsal eyes, represent promising candidates to test the role of *spineless* and the Iroquois complex in regional specialization in the context of sexual dimorphism (Sison-Mangus et al. 2006). Dorsal–ventral differences in filter pigment distribution are widespread in butterflies. Investigating how filter pigments are regulated during dorsal–ventral patterning, and comparing these processes to pigment regulation in stochastic ommatidial differentiation, may provide insights into broader mechanisms of tissue patterning.

7. Spectral sensitivity and color vision

Photoreceptor spectral sensitivity is shaped by a combination of molecular and optical features (Fig. 6), including opsin gene duplication and divergence, spectral tuning of opsin protein sequences, co-expression of multiple opsins, and lateral filtering by perirhabdomal pigments (van der Kooi et al. 2021; Mulhair et al. 2023). In many butterfly lineages, these mechanisms combine to produce extreme photoreceptor diversity (Arikawa et al. 1987; Ogawa et al. 2013; Chen et al. 2013, 2016; McCulloch et al. 2017; Blake et al. 2019). For example, in *Graphium sarpedon*, as many as 15 distinct spectral sensitivities have been identified due to a combination of multiple opsin duplications and distinct lateral filtering pigments (Pirih et al. 2022)

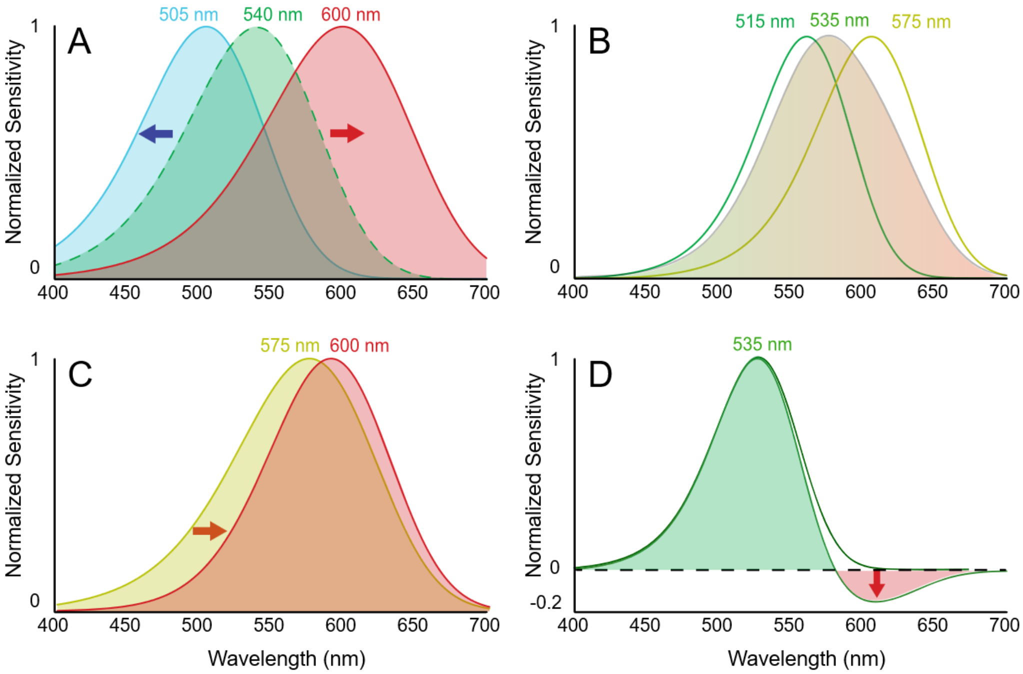

Figure 6. Mechanisms that modify photoreceptor spectral sensitivity

(A) *Gene duplication and divergence.* In *Apodemia mormo*, an ancestral LW opsin underwent duplication. The two resulting copies have since accumulated amino acid substitutions, producing a red-shifted opsin and a blue-shifted opsin (Frentiu et al. 2007). **(B)** *Opsin co-expression.* In *Papilio xuthus*, co-expression of opsins L2 and L3 generates a broadband photoreceptor with peak sensitivity around 535 nm (Arikawa et al. 2003). **(C)** *Lateral filtering.* In *Papilio xuthus*, the proximal R5–8 photoreceptors in type I ommatidia express L3 (λ_{max} 575 nm). Due to the lateral filtering effect of red perirhabdomal pigments, these red photoreceptors exhibit a narrower spectral bandwidth and a red-shifted peak sensitivity of approximately 600 nm (Arikawa et al. 1999). **(D)** *Direct inhibition.* In *Charaxes jasius*, green photoreceptors that receive direct inhibitory input from red photoreceptors (λmax 620 nm) retain their peak sensitivity at 535 nm, but display a narrower spectral bandwidth and a hyperpolarizing response in the red wavelength region (Belušič et al. 2021).

Color vision depends on both photoreceptor diversity and the neural circuits that compare signals from these diverse photoreceptors (Schnaitmann et al. 2020). Such comparisons are encoded by color-opponent neurons that exhibit excitation at certain wavelengths and inhibition at others. In *Drosophila*, color-opponent processing occurs as early as the photoreceptor stage, where direct inhibitory synapses only form between the long visual fibers of R7 and R8 photoreceptors in optic chiasm or medulla (Schnaitmann et al. 2018; Kind et al. 2021). In *Papilio* butterflies, however, extensive inter-photoreceptor inhibitions exist among long visual fibers (R1/2) and short visual fibers (R3-8 and R9) within the lamina, contributing to the spectrally complex visual system (Matsushita et al. 2022). These photoreceptors with spectral opponency have also been recorded in other Papilionidae and Nymphalidae species (Chen et al. 2013, 2020a; Belušič et al. 2021; Ilić et al. 2022; Pirih et al. 2022; VanKuren et al. 2025).

Despite the high diversity of photoreceptor types in Lepidoptera, not all contribute to color opponency or color vision. In the tetrachromatic *Papilio xuthus*, the minimum discriminable wavelength difference function exhibits three minima, indicating that only four classes of receptors contribute to color vision, despite the presence of at least eight distinct spectral sensitivity types (Koshitaka et al. 2008). The photoreceptors not contributing to tetrachromacy are all confined to type II ommatidia (Koshitaka et al. 2008). These excluded photoreceptors are likely specialized for non-chromatic functions, such as motion detection or polarization vision. For instance, R3/4 in *P. xuthus* exhibit the fastest response latencies among photoreceptors (Kawasaki et al. 2015), a characteristic that may facilitate motion detection using chromatic contrast (Stewart et al. 2015).

8. Non-photoreceptor cells in compound eye

Retinal development has been well characterized in *Drosophila melanogaster*, where the adult compound eye arises from a monolayer of undifferentiated epithelium known as the eye-antennal disc (Kumar 2012). During larval and pupal stages, photoreceptor neurons are specified first, followed by the recruitment of cone cells and primary pigment cells. Cells that do not adopt one of these fates subsequently differentiate into secondary or tertiary pigment cells (Kumar 2012). In Lepidoptera, retinal development likely follows a similar sequence, at least for photoreceptor recruitment (Gao et al. 2025). Our current understanding of non-photoreceptor cells in Lepidoptera is primarily based on ultrastructure studies using electron microscopy.

Each ommatidium typically has four cone cells, two primary pigment cells (PPCs), and six secondary pigment cells (SPCs), which are shared between adjacent ommatidia (Ribi 1978; Kolb 1985). Cone cells secrete the dioptric apparatus, including cornea and crystalline cone. In *Drosophila*, they can also direct cell type differentiation during ommatidia development and support homeostasis in adult photoreceptors (Charlton-Perkins et al. 2017, 2021). In *Pieris*, the PPCs envelop the cone cells and the distal half of the crystalline cone. These pigment cells help regulate light influx by contracting distally during light adaptation. SPCs cover the proximal half of the crystalline cone and the photoreceptors down to the basement membrane, shielding stray light from nearby ommatidia. During light adaptation, pigment granules in SPCs accumulate in the distal region. Another set of pigment cells, basal pigment cells (BPCs), are located below the basement membrane. These cells insulate photoreceptor axons and, together with SPCs, form the dense pigmentation layer at the base of the ommatidium (Ribi 1978). BPC pigment granules differ significantly in size compared to those of PPCs, SPCs, and photoreceptors (Fischer et al. 2012). This suggests that the subretinal pigment layer consists of a novel pigment cell type. In other insects, the subretinal pigment layer is derived from secondary/tertiary pigment cells (Tomlinson 2012) or lateral rim pigment cells (Mohr et al. 2020). Whether BPCs originate from subretinal or retinal tissue remains unclear. Comparative transcriptomic analysis with PPCs, SPCs, and other subretinal glial cells may help resolve their developmental origin.

In Lepidoptera, tracheal cells form a reflective structure known as the tapetum at the base of the rhabdom. In nocturnal moths, the tapetum consists of numerous tracheoles with alternating air and cytoplasm, forming an interference reflector that mirrors unabsorbed light back through the rhabdom. The tapetum is located just above the basement membrane. In many diurnal butterflies, this ancestral tracheal tapetum has been modified into a few branches at the proximal end of the rhabdom, generating color eyeshine (Ribi 1979). The tapetum has been independently lost at least twice in butterflies: once in the family Papilionidae and once in the genus *Anthocharis* (Takemura et al. 2007).

9. Conclusions and future directions

The compound eyes of butterflies and moths exhibit remarkable diversity in cellular composition, spectral tuning, and spatial organization. The spectral diversity arises from a combination of opsin gene duplication and divergence, opsin co-expression, lateral filtering, and direct inhibition among photoreceptors. Different lineages, such as Papilionidae, Lycaenidae, and Nymphalidae, have evolved distinct mechanisms to expand sensitivity into the long-wavelength range, through a combination of red filter pigments, LW opsin duplication and divergence, and green-shifted B opsin. These innovations, along with regional specializations like dorsal–ventral patterning and polarization-sensitive DRA ommatidia, reflect tight coordination among anatomical, molecular, and functional components of the eye. For example, in *Papilio xuthus*, five opsins and three types of filtering pigments are integrated into just three ommatidial types.

We have only begun to understand the developmental mechanisms that underlie this precisely regulated coordination of the various features of ommatidia (Perry et al. 2016).

Despite progress in characterizing eye structure and photoreceptor diversity, many fundamental questions remain. The molecular logic underlying complex retinal mosaics, particularly in species with more than three ommatidial types like *Heliconius*, is still unknown. Similarly, the developmental origin and function of lesser-known cell types, such as basal pigment cells, and the role of photoreceptors not involved in color vision remain poorly understood. These photoreceptors may contribute to spatial and motion vision, or even wavelength-specific behaviors that are ecologically important but largely unexplored. Additionally, the rapid turnover in eye designs across Lepidoptera, including repeated shifts between apposition and superposition eyes, raises questions about how intermediate forms remain functional. Understanding the impact of opsin or filtering pigment changes on downstream visual circuits will be key to understanding how color processing is preserved or reshaped during evolution.

To address these gaps, future research should focus on three main areas. First, identifying the ecological pressures that drive visual system diversification will clarify the adaptive value of specific photoreceptor types and spectral sensitivities. Second, dissecting the genetic and regulatory basis of compound eye variation, especially with the help of comparative genomics and single-cell multi-omics, will illuminate how new eye designs evolve and what constraints shape them. Finally, much remains to be learned about visual processing circuits in butterflies and moths, particularly given their unique features like the multi-tiered rhabdom structure, diverse opsin and pigment variation in R1-8, and the R9 photoreceptor projecting to the lamina. Understanding how these differences influence color perception and behavior will provide deeper insight into the evolution of sensory systems more broadly.

Supplemental Table S1

Summary of opsin gene copy numbers in Lepidoptera species.

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