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Looking across the gap: Understanding the evolution of eyes and vision among insects

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Abstract

The compound eyes of insects exhibit stunning variation in size, structure, and function, which has allowed these animals to use their vision to adapt to a huge range of different environments and lifestyles, and evolve complex behaviors. Much of our knowledge of eye development has been learned from *Drosophila*, while visual adaptations and behaviors are often more striking and better understood from studies of other insects. However, recent studies in *Drosophila* and other insects, including bees, beetles, and butterflies, have begun to address this gap by revealing the genetic and developmental bases of differences in eye morphology and key new aspects of compound eye structure and function. Furthermore, technical advances have facilitated the generation of high-resolution connectomic data from different insect species that enhances our understanding of visual information processing, and the impact of changes in these processes on the evolution of vision and behavior. Here, we review these recent breakthroughs and propose that future integrated research from the development to function of visual systems within and among insect species represents a great opportunity to understand the remarkable diversification of insect eyes and vision.

KEYWORDS
compound eye, development, evolution, insects, vision

INTRODUCTION

Insects have adapted to almost all terrestrial environments on earth and exhibit complex behaviors and life history strategies. This success can at least partially be attributed to the enormous diversity they exhibit in their compound eyes and vision. While vision and behavior are investigated in a wide range of insect species, eye developmental processes that underpin the evolution of insect eyes have predominantly been analyzed in *Drosophila melanogaster* due to its vast repertoire of genetic tools. Here, we briefly review insect eye structure, diversity, and formation as a platform to then highlight recent advances in understanding the evolution of eye development and function in *Drosophila* and crucially in other insect species that represent the incredible diversity in eyes and vision found across these animals. This combination of ever-advancing new tools and knowledge from *Drosophila* with new insights into visual systems of a growing range of other species is closing the gap in our understanding of insect eyes and vision by providing a more complete picture of their evolution and function.

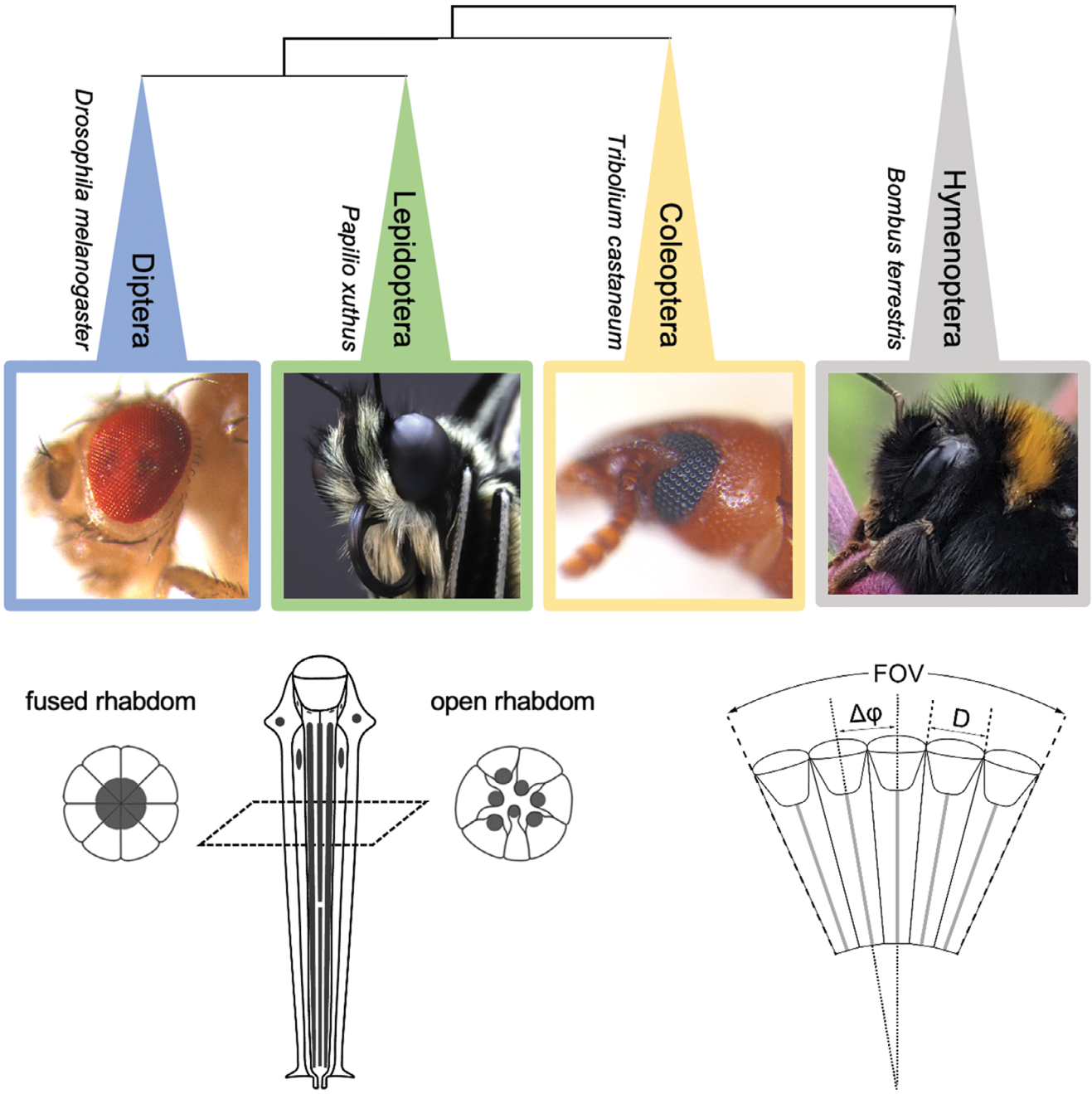

FIGURE 1 Compound eyes in holometabolous insects. Examples of the head and compound eyes of representatives of four major orders of holometabolous insects (top). Schematic of an ommatidium with cross section views of open and closed rhabdom configurations illustrated (bottom left). Schematic of ommatidia indicating field of view (FOV), inter-ommatidial angle ($\Delta\phi$), and ommatidium diameter (D) (bottom right). Pictures were kindly provided by Markus Friedrich (*T. castaneum*), Kentaro Arikawa and Michiyo Kinoshita (*P. xuthus*), Vivek Nityananda (*B. terrestris*), and Javier Figueras Jimenez (*D. melanogaster*).

Insect compound eye structure and diversity

Insect compound eyes are made up of optical units called ommatidia. Photons are focused through the corneal lenses onto the underlying photoreceptor cells (PRCs), specifically their rhabdomeres that contain rhodopsins (Figure 1; Box 1). A major source of the great diversity in insect eyes is differences in ommatidia number, diameter, and length. The number of ommatidia can vary hugely between species, for example, from 29 in a parasitoid wasp[1] to tens of thousands in dragonflies.[2] There is also extensive natural variation within species; the eyes of *D. melanogaster* strains, for example, can differ by hundreds of ommatidia.[3] Ommatidia diameter also varies considerably among species, within species and even across different regions of the eyes of individuals.[4–7] For example, male *Chrysomya megacephala* have wide ommatidia in the dorsal two thirds of the eye and much narrower ommatidia in the ventral third.[8] Differences in the relative number

Box 1: Glossary of Terms

Acuity
Measure of the ability of eyes to resolve different objects.

Apposition eyes
Compound eyes with fused rhabdoms where the photoreceptors collect light from a single ommatidial lens and project to one laminal cartridge.

Connectomics
Mapping of the axonal connections between neurons in the nervous system, for example, in the optic lobe neuropils of insects.

Contrast sensitivity
Measure of the ability of eyes to detect patterns with decreasing contrast.

Inter-ommatidial angle
The angle between ommatidia along their optic axes.

Laminal cartridge
Column of the lamina underlying a single ommatidium.

Lamina monopolar cells
Laminal neurons that collect information from photoreceptors and connect to other cartridges and the medulla.

Microsaccades
Rapid movements of the eyes that shift the fixed point of gaze, achieved by moving the whole head or retinas. Photoreceptor microsaccades are the contraction of individual photoreceptors in response to light.

Neural superposition eyes
Apposition eyes with open rhabdoms where photoreceptors with the same field of view project axons to the same laminal cartridge, and photoreceptors within an ommatidia with different fields of view project to different cartridges.

Ommatidia
Subunits that make up the compound eyes that are composed of a single lens, photoreceptor cells, cone cells and accessory cells including pigment cells.

Optic neuropils
Subunits of the optic lobe; the lamina, medulla, lobula, and lobula plate.

Photoreceptor cell
Light sensitive cells in the ommatidia of compound eyes that express rhodopsins in specialized rhabdomeres.

Rhabdom
The group of rhabdomeres within a single ommatidia, which can be arranged in an open or fused configuration.

Rhabdomere
Specialized photoreceptor cell sub-structure with a large surface area made up of thousands of microvilli presenting rhodopsins.

Rhodopsins
Photosensitive molecules localized to rhabdomeres and responsive to different wavelengths of light. Rhodopsins are composed of a G-protein coupled transmembrane receptor (opsin) covalently bound to the chromophore ligand (retinal).

Superposition eyes
Compound eyes with fused rhabdoms where photoreceptor cells with the same field of view collect light from multiple ommatidial lenses to enhance light detection.

and width of ommatidia often indicate regions with specialist functions, for example, larger frontal ommatidia of killer flies that hunt in flight.[7] Even within the eye of *D. mauritiana* we have observed facet size differences up to 35%.[5] Ommatidia length also differs considerably within and between species, for example, rhabdomere length can vary from 0.46 to 1.1 mm within the eye of a dragonfly.[9,10] Differences in ommatidia number, diameter, and length affect the inter-ommatidial angles and determine how much light enters each ommatidium thus directly affecting acuity and contrast sensitivity[11–13] (Figure 1; Box 1). Additionally, these parameters and the overall field of view vary with the curvature of the eye[11,12] (Figure 1).

The PRCs in each ommatidium express photo-sensitive rhodopsins (Rh) (Box 1) that can respond to different wavelengths of light. In *Drosophila*, the six outer PRCs (R1–R6) in each ommatidium express Rh1 and are involved in motion detection, while the two inner PRCs (R7 and R8) detect UV (Rh3 and RH4), blue (Rh5), or green (Rh6) light depending on which rhodopsins they express (Figure 2) and thus define different ommatidial subtypes.[14,15] The evolution of rhodopsin

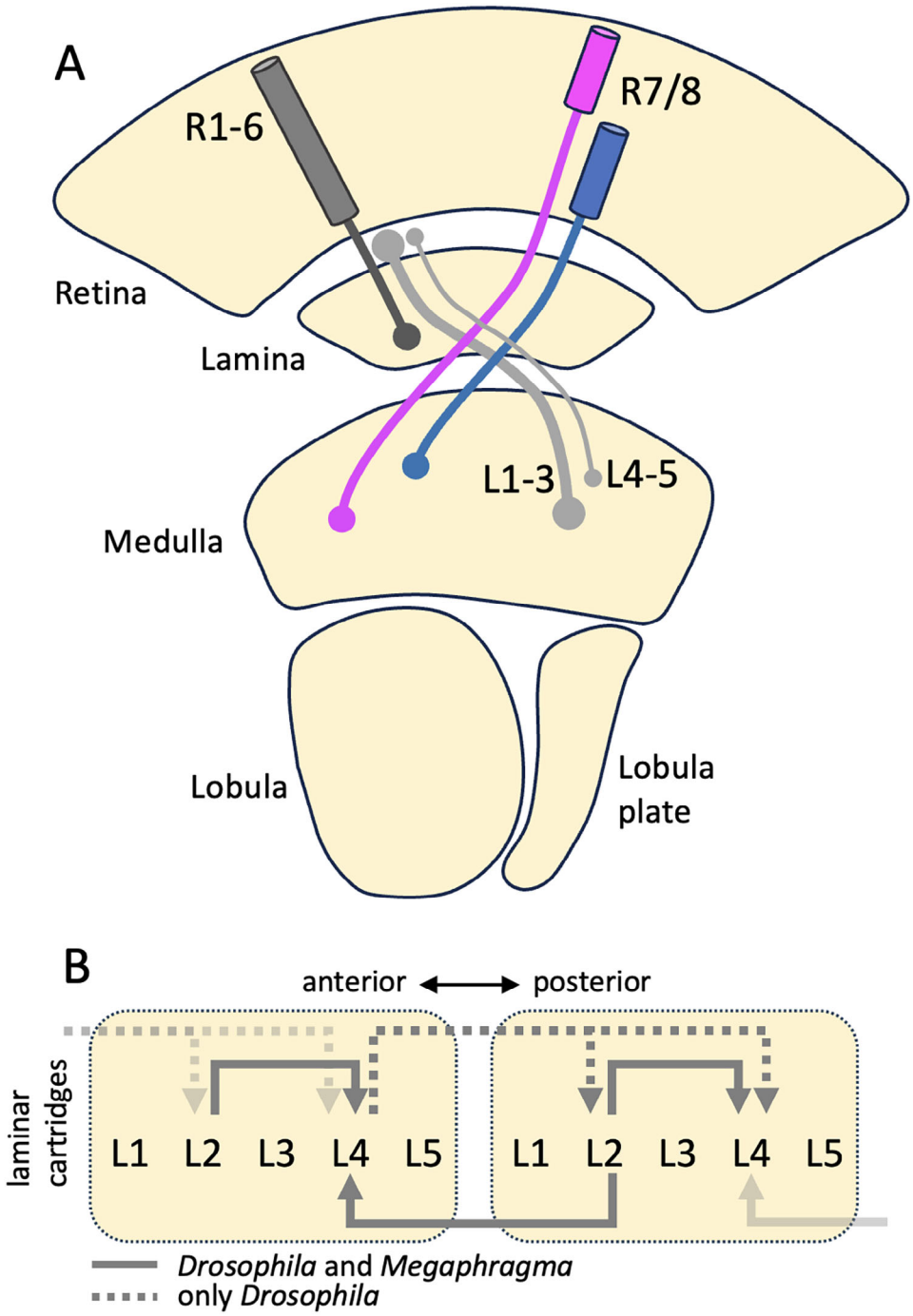

FIGURE 2 Neuropils and connections in the optic lobes of *Drosophila melanogaster*. (A) The four neuropils of *D. melanogaster* showing selected major connections between the retina, lamina, and medulla. (B) Connections between lamina monopolar cells (L1–L5) in adjacent lamina cartridges in *D. melanogaster* and the parasitoid wasp *Megaphragma viggianii*.[1]

repertoires and their expression underlies differences in spectral sensitivities, color vision, and motion detection within and among insect species[15–18] (Box 2). For example, it was recently shown that sex-linkage of *UVRh1* underlies differential UV sensitivity between male and female *Heliconius* butterflies.[19]

Ommatidial subtypes can be found randomly distributed across the eyes of insects, for example, the "pale" (R7 expressing RH3, R8 expressing RH5) and "yellow" (R7 expressing RH4, R8 expressing RH6) ommatidia subtypes of *Drosophila*, or more regionalized and even in stripes or bands (reviewed in ref. [15]). Similar organizational principles are evident across insects, however, including the specialized dorsal rim area (DRA) and there are potentially conserved aspects of the regulation of rhodopsin expression and the specification and distribution of ommatidial subtypes.[15,20,21]

Signals from the PRCs are transmitted to dedicated inter-connected neuropils of the optic lobe (lamina, medulla, lobula, and lobula plate)

Box 2: Suggested Selected Further Reading

Almudi et al., 2019.[24]
Describes the establishment of *Cloeon dipterum*, a mayfly, as model system with great potential to better understand insect eye evolution, development and function.
Bollepogu Raja et al., 2023.[108]
Single-cell analysis of gene expression and chromatin profiling of cell types in *Drosophila* larval eye discs.
Chen and Desplan, 2020.[39]
Overview of the components and interactions of the gene regulatory networks that build different parts of the *Drosophila* visual system.
Friedrich, 2006.[37]
Key review of the evolution and development of eyes in insects, comparing the fly with beetle and grasshopper.
Gonzalez-Bellido et al., 2022.[58]
Overview of the extensive range of visual adaptations of predatory arthropods including insects.
Heras and Laughlin, 2024.[9]
Modeling of relative investment in photoreceptors and eye optics that have influenced insect apposition eye design. Includes a summary of measurements of different regions of neural superposition and fused rhabdom eyes of ten insect species from previously published work.
Kinoshita and Arikawa, 2023.[62]
Recent review of color vision adaptations in butterflies focusing on the swallowtail butterfly *Papilio xuthus*.
Konstantinides et al., 2022.[104]
Temporal single-cell analysis of expression of the transcription factors that specify the neurons in the optic lobes of *Drosophila*.
Land 1997.[12]
Seminal review of visual acuity in insects including tables of eye types and interommatidial angles of a range of insects.
McCulloch et al., 2022.[17]
Reviews the regulation of rhodopsin expression and evolution of rhodopsin repertoires across insects.
Meece et al., 2021.[59]
Overview of visual adaptations of a range of insects and other arthropods including dragonflies, butterflies and spiders.
Shinomiya et al., 2022.[83]
3D reconstruction of the connectivity of neural circuits for computation of visual motion in the lobula plate of *Drosophila*, which exemplifies how connectomics can help understand the processing of visual information in the optic lobes.
Song and Lee, 2018.[61]
Overview of insect color vision from spectral sensitivity of photoreceptors and processing of chromatic information to ecology and behavior.
Warrant, 2017.[23]
Review of the visual adaptations of nocturnal insects.
Warrant and Somanathan, 2022.[60]
Comprehensive recent overview of an example of a visual adaptation in insects – the color vision of nocturnal insects.
Warren and Kumar, 2023.[28]
Recent review discussing the genetic regulation and cellular processes underlying *Drosophila* eye development focusing on the morphogenetic furrow and how this differentiation wave triggers formation of ommatidia.
Wernet et al., 2015.[15]
Comprehensively reviews the regulation of photoreceptor specification and rhodopsin expression in the *Drosophila* retinal mosaic and in the eyes of other insects.

(Figure 2; Box 1) that harbor complex networks of tens of thousands of neurons that process and transmit this information to the central brain to regulate behavior. In *Drosophila*, the outer PRCs from each ommatidium send axons to the lamina whereas inner PRCs project to the medulla. The signals are then passed on to the higher-level processing centers—the lobula and lobula plate—for color and polarized light vision and/or motion detection respectively (reviewed in ref. [14]) (Figure 2).

Most insects have apposition eyes (Box 1) where ommatidia are insulated from each other by pigment and the rhabdomeres of the PRCs are fused (fused rhabdom, Box 1). Information from a single ommatidium is transmitted via an axon "bundle" to the same

laminal cartridge in the optic lobe[22] (Figures 1 and 2). This arrangement maximizes the spatial resolution that can be achieved by large numbers of ommatidia in diurnal insects.[11,22] In contrast, many nocturnal insects have superposition eyes (Box 1), where a "clear zone" between the lenses and the rhabdoms allows a single PRC to detect photons collected by multiple neighboring lenses.[23] This arrangement maximizes sensitivity in dim light at the cost of resolution.[23] Dipterans have evolved neural superposition eyes (Box 1), which are apposition eyes with separated ommatidia but open rhabdoms (Figure 1; Box 1), where axons from PRCs of different ommatidia but overlapping fields of view project to the same laminal cartridge (Box 1), while individual PRCs within an ommatidium with different fields of view project to different cartridges (Figure 2). This arrangement is thought to enhance sensitivity while also maximizing resolution.[22]

Recent modeling and analysis of morphological data of insect neural superposition and fused rhabdom apposition eye have shown that the competing costs of photoreceptors and optics greatly influence eye design and evolution, and visual ability, with investment in the former often exceeding the latter[9] (Box 2). However, detailed comparative analysis of eye structure and visual performance are needed across different regions of the eyes of a wider range of species to better understand their evolution and how this is influenced by costs and other potential constraints.[9]

Development of insect compound eyes

A major challenge in understanding the evolution of many visual system adaptations is studying the mechanisms underlying their development. While field-collected adult insects can be used directly in experiments to study vision, characterizing the development of their eyes requires analysis of earlier developmental stages that may be inaccessible in the wild and/or currently difficult, if not impossible, to culture in the laboratory. However, great progress has been made in establishing exciting new models for the evolution of eye development and vision, including mayflies, where the males have an extra pair of turbinate eyes used to identify females during flight[24,25] (Box 2), and the diving beetle *Thermonectus marmoratus*, which has evolved superposition eyes and thus increased light sensitivity.[26]

While all insect compound eyes likely have the same evolutionary origin,[27] their basic development differs among lineages. *Drosophila* eyes develop from a pair of internalized eye-antennal imaginal discs that grow through the first two larval instars before differentiating during the third instar and then fusing during metamorphosis in the pupal stage[28–30] (Box 2). Although this process was already well-understood,[28,31] Navarro et al. (2024)[32] recently provided important new insights into how cell division, differentiation, and cell death are coordinated in the developing eye disc of *Drosophila* to specify cell number and eye size.

In contrast to *Drosophila*, most other insects do not have internalized eye imaginal discs and instead their eyes develop more directly from eye primordia in the larval head. In the beetle *Tribolium castaneum*, for example, the eyes develop from epithelia that are part of the lateral head of the larva.[33,34] Similarly in the honeybee, *Apis mellifera*, the eyes develop from placode-like epithelia visible from the third instar on either side of the head, flanking the optic lobe primordia, and ommatidia differentiate from pre-ommatidial clusters during the fifth instar.[35,36] Similar to *Drosophila*, the differentiation of the ommatidia in both *Tribolium* and *Apis* proceeds from posterior to anterior, suggesting a shared ancestral mechanism for retinal differentiation[36,37] (Box 2). However, further systematic analysis of the cellular processes that build the eyes of holometabolous insects like *Tribolium* and *Apis* as well as a much wider range of insects, including direct developers, is sorely needed to understand the development and evolution of insect eyes more generally.

Establishing the genetic regulation of compound eye development and how this is conserved or divergent across insect species is crucial to understanding the evolution of insect eye diversity. The eye gene regulatory network (GRN) is very well understood in *Drosophila*: eyes are specified by the *Pax6* genes *eyeless* (*ey*) and *twin of eyeless*, which act upstream of other retinal determination genes including *sine oculus* and *eyes absent*[28,30,31,38,39] (Box 2). Building on knowledge from *Drosophila*, candidate gene studies in other insects suggest that eye development is regulated by a similar suite of retinal determination genes among insects, although there are differences in the spatial and temporal deployment, and in the interactions, of some of these factors.[27,33,37,40–42] (Box 2). Therefore there is likely very much to be learned from studying the genetics underpining the regulation of eye development in a wider range of insects.

DECIPHERING THE GENETIC BASIS OF INSECT EYE MORPHOLOGY EVOLUTION

The great diversity of eye size and structural composition among insects begs the question of what are the genetic and developmental bases of these evolutionary changes? *Drosophila* is an excellent system to address this question because of the depth of knowledge of the genetic and cellular mechanisms that regulate eye development in *D. melanogaster*[38] and substantial differences in the eye morphology among *Drosophila* species.[3,43–46]

Ramaekers et al. (2019)[47] showed that a single nucleotide change in a binding site for the transcription factor Cut in an enhancer of the *Drosophila Pax6* gene *eyeless* (*ey*) underlies ommatidia number and consequently eye size differences between strains of *D. melanogaster* and this mechanism likely contributes to the eye size difference between *D. melanogaster* and *D. pseudoobscura*.[47] Furthermore, differences in ommatidia number between *D. melanogaster* and *D. simulans* are also associated with sequence changes in this Cut binding site in the enhancer of *ey*.[3] This suggests that changes in *ey* regulation could be an evolutionary hotspot, at least among *Drosophila* species, and it would be interesting to test whether this regulatory mechanism is involved in eye size differences in other insects. However, differences in *Drosophila* ommatidia number have been found to be polygenic[3,48,49]. Consistent with this, the nucleotide change in *ey* alone does not explain the full ommatidia number difference in the focal strains studied by

Ramaekers et al. (2019),[47] and this nucleotide is the same in other strains of *D. melanogaster* and *D. simulans* with intra-specific variation in ommatidium number.[3] Interestingly these intra-specific differences in eye size also appear to be caused by different developmental mechanisms including relative timing and rates of cell division versus differentiation.[3] In addition, Buchberger et al. (2021) showed that the gene *pannier* acts after Cut regulation of *ey* during sub-division of the eye-antennal disc to generate differences in ommatidia number and overall head shape between *D. melanogaster* and *D. mauritiana*.[50]

Much less is understood about the development of ommatidia diameter differences within eyes, even in *Drosophila* (but see[51]), never mind how this critical aspect of eye morphology evolves between species. It was recently shown that the wider ommatidia of *D. mauritiana* compared to its sibling species *D. simulans* is associated with changes in the expression of the transcription factor Orthodenticle (Otd) as the ommatidia mature.[4,44,52] Otd is a highly conserved transcription factor in insect eye development[41] and uncovering the downstream mechanism in these *Drosophila* species could help understand the regulation and evolution of ommatidia size among insects more broadly.

The studies above demonstrate the enormous potential to decipher the genetic basis of differences in eye size and structure among other *Drosophila* species[43,45,48] and this could help to pinpoint the nodes of evolution to better understand how GRNs evolve more generally. While much less is known in other insects, models generated for eye development such as beetles, bees, and butterflies are already demonstrating their great potential to identify and investigate candidate genes to test mechanisms from *Drosophila* and more fully explain diversification of eye morphology among insects.

Rathore et al. (2023)[26] compared the function of the transcription factor Cut between *D. melanogaster* with neural superposition eyes and the diving beetle *T. marmoratus* with optical superposition eyes using RNAi knockdown. They found that *cut* likely plays similar roles in ommatidium formation in these two insects including regulating development of the cone (Semper) cells, important support cells that secrete the lens.[26] This evidences the importance of cone cells and the conserved role of Cut in regulating ommatidia formation among insects with different optics.

Recently, Netschitailo et al. (2023)[53] revealed how the sex determination pathway regulates eye size dimorphism in honeybees. The eyes of male honeybees (drones) are four times larger with increased ommatidium diameters compared to females, which is an adaptation to identify queens during mating flights.[54–56] They identified *glubschauge* (*glu*) as a sex-specifically spliced transcription factor gene with only the female protein isoform (*gluF*) containing a zinc finger DNA-binding domain. Knockout of *glu* in females using CRISPR/Cas9 increased eye size, while overexpression of *gluF* in males reduced ommatidia size and overall eye size. Identifying the regulatory mechanisms of *glu* as well as the target genes of GluF offers an exciting opportunity to understand the role of the sex determination pathway and alternative splicing in generating different eye morphologies in male and female bees. Intriguingly, *glu* orthologs are found in other insects but are not alternatively spliced and lack the zinc finger domain.[53] Therefore, this could represent a novel mechanism specific to bees. However, sexual dimorphism in eye size is pervasive in insects, including *Drosophila*. Which components of the sex determination pathway are involved in differences in eye morphology in other insects and if they act through similar or distinctive developmental mechanisms and downstream targets in eye development GRNs remains an open and interesting question to pursue.

THE FUNCTIONAL IMPACT OF VARIATION IN EYE MORPHOLOGY ON VISION

The astonishing behavior of many insects is only possible because of their underlying visual capabilities. While this has been reviewed thoroughly elsewhere[11,22,57–62] (Box 2) the following recent examples serve to illustrate this point.

The remarkable flight acrobatics of dragonflies rely on the amazing visual systems these aerial predators have evolved (reviewed in ref. [63]). The eyes of dragonflies are fused dorsally while those of the related damselflies are separated with binocular overlap. This difference in the eye arrangements as well as the position of target-selective descending neurons reflects the different hunting strategies of these two predators: dragonflies target their prey dorsally while damselflies approach from the front using their binocular vision.[64] The visual systems of many insects have also evolved to facilitate nocturnal behaviors including superposition eyes (see above). Indeed, some insects such as giant hawkmoths (reviewed in ref. [60]) and the Asian giant honeybee *Apis dorsata*[65] even have color vision at night.

The broad patterns of the evolution of the major insect eye types and how they impact vision and may underlie adaptations are reasonably well understood. However, it is often difficult to identify the underlying mechanisms and evolutionary drivers across deep macroevolutionary timescales. Therefore, a better understanding of the impact of more recent evolutionary differences in eye morphology on vision within species and between closely related species is needed to both decipher proximal events and inform understanding of more ancient differences.[66] Several recent studies have shown that this can be a powerful approach to understand eye structure-function links and in some cases even why differences may have been selected during the course of evolution.

We recently modeled and tested differences in vision between *D. mauritiana* and *D. simulans* resulting from the wider ommatidia of the former species.[5] As predicted, the behavioral experiments showed that *D. mauritiana* eyes have greater contrast sensitivity while those of *D. simulans* have enhanced acuity. While *D. simulans* is a cosmopolitan generalist, *D. mauritiana* is restricted to Mauritius and this finding may help to better understand their ecology and behavior, and why these differences may have been selected.

Analysis of eye size differences between other *Drosophila* species has provided insights into their behavioral differences. *D. subobscura* has 25%–30% more ommatidia and larger optic lobes than the sympatric *D. pseudoobscura*, which has larger antennae and antennal

lobes.[45] This is consistent with a trade-off between visual and olfactory sensory systems described more widely in other fly species.[43,47] The mating of *D. subobscura* is more light-dependent and therefore more visual than that of *D. pseudoobscura* and the differences in eye size between these two species may be related to niche partitioning, with *D. subobscura* inhabiting lighter environments than *D. pseudoobscura*.[45] Investigating the genetic and developmental bases of this difference in eye and optic lobe size between *D. subobscura* and *D. pseudoobscura* would likely provide valuable new insights into the evolutionary diversification of *Drosophila* eyes.

In the future, it would also be interesting to more broadly compare the changes in eye development, structure, and vision among *Drosophila* species to those among other closely related insects to study whether similar or different mechanisms are employed across lineages. *Heliconius* butterflies represent a very promising system to do this. *Heliconius cydno* inhabits dense forests whereas the closely related *H. melpomene* is found around the forest edges in more open habitats.[67] *H. cydno* has larger eyes, composed of more ommatidia, and bigger visual neuropil volume compared to *H. melpomene* and these morphological differences are under directional selection.[68–70] The males of both species also have larger eyes than the females.[68,70] These differences are presumably adaptations to the different habitats they occupy and for males to find mates.[68] Interestingly the eyes of the hybrid F1s of these two species are similar to *H. melpomene* while their neuropils are similar to *H. cydno* or intermediate between the species.[69] This exciting finding suggests there are differences in the coordination of eye and optic lobe development between these species, which disrupts hybrid visual systems and may affect their fitness.[68,69] This system has great potential to identify the genes underlying these differences in eye morphology and coordination with optic lobe development because these butterflies can be cultured and genetic mapping is possible (e.g.[71]). Furthermore, the visual abilities of *Heliconius* species and their F1 hybrids could be investigated to determine the consequences of differences in optical parameters and visual information processing. This would provide a unique connection between genetics, eye development, morphology, and visual behavior and ecology in butterflies.

In *Drosophila* and other insects, eye size is often linked to variation in body size, which subsequently has implications for the number and size of ommatidia and therefore optic parameters. Recent papers have described striking natural variation in eye size within and between bumblebee species that are predicted to cause differences in their vision based on morphological measurements.[72,73] This plasticity in eye development within bumblebees has been suggested to produce workers with different visual abilities that allows optimal foraging in alternative niches with varying light conditions,[72] therefore facilitating microhabitat niche partitioning within species, as suggested above for partitioning between fly and butterfly species.[73]

A new study of the hummingbird hawkmoth *Macroglossum stellatarum* has shown that the superposition eyes of this insect scale hypoallometrically with body size and that smaller hawkmoths have fewer but wider ommatidia.[74] This allows these insects to maintain the balance between acuity and contrast sensitivity despite differences in body size.[74] Future studies focusing on the underlying developmental mechanisms have great potential to better understand eye size plasticity and evolution across insects.

ACTIVE VISION

Compound eye structure alone does not fully explain visual ability and so we need to consider other aspects of eye function and information processing to understand the evolution of visual systems across insects. Most insects cannot move their eyes independently of their head to shift gaze or generate optic flow for spatial information. They must therefore rotate their entire head or body.[75–77] Several recent studies have provided new insights into the structure and functionality of *D. melanogaster* eyes that affect their active vision with implications for understanding the quality of visual information that insects can perceive.

It was previously observed in the housefly *Musca domestica* that the retina was served by two muscles that could move it in response to external cues[78] and thus move PRCs relative to the lens while the head remained still.[78] Fenk et al. (2022)[79] recently described and investigated the function of these muscles, *musculus orbito tentoralis* and *musculus orbito scapalis*, in *D. melanogaster*. Optogenetic activation of the motor neurons attached to these muscles resulted in approximately 15° of movement of the PRCs. Importantly, this movement did not affect the alignment of PRCs in adjacent ommatidia necessary for neural superposition. The results suggest that these muscles allow the fly to move its retina in two directions to stabilize gaze when tracking a moving environment (similar to human eyes) in concert with head and body movements.

Fenk et al. (2022)[79] found that these retinal movements were used during gap crossing and this ability was perturbed when signaling of retinal motor neurons was blocked. This suggests that the retinal dynamics are involved in a "binocular ruler mechanism" and the authors conclude that these results indicate that the retinal movements allow flies to realign their PRCs to better visualize fine-scale features and may be involved in spatial attention and object recognition.[79] This exciting discovery complements recent further characterization of PRC microsaccades (Box 1) in *D. melanogaster*.

Kemppainen et al. (2022)[80] showed that previously observed[81] PRC contractions in response to light (PRC microsaccades, Box 1) sweep across both eyes mirror symmetrically in *D. melanogaster*. Interestingly, stimulating one PRC in an ommatidium caused all its other PRCs to move but not the PRCs in neighboring ommatidia, which suggests these movements are not caused by the retinal muscles described above. In fact, the findings of Kemppainen et al. (2022)[80] suggest the PRC microsaccades are photomechanical and induced by phototransduction. Moreover, the symmetrical movement suggests that PRCs in the left and right eyes with overlapping frontal fields are both scanning in the stereo-range. Models and simulations predict that this results in better resolution and stereopsis than predicted by classic models.[80] Kemppainen et al. (2022)[80] experimentally demonstrated hyperacute stereopsis in *Drosophila* using salience and learning

experiments and showed that flies can detect 3D objects and 2D shapes at higher resolution than predicted by static morphology. Interestingly, PRC microsaccades were also recently recorded in the fused rhabdom eyes of honeybees implying that these are a conserved feature of insect compound eyes.[82]

Together these two recent key studies[79,80] show that the visual ability of *Drosophila* eyes is more complex than previously understood and by what can be inferred from their static morphology alone. This has profound implications for understanding insect compound eye function and vision. It will be important to identify and characterize retinal movements and PRC microsaccades within and between other insect species to complement studies of their static eye morphology. Variation in these factors is likely to impact visual ability and could help insects adapt to different environments and affect their behavior.

INFORMATION PROCESSING

Recent advances in connectomics (Box 1) of the *Drosophila* brain and visual system, computational modeling, and behavioral approaches have been utilized to map neuronal projections and describe the structure of the optic lobe neuropils to better understand the neural computation underlying processing of visual information (e.g.[83–87]) (Box 2). This has provided unprecedented new insights into color vision, motion detection, movement, and navigation in this fly (Box 2). For example, flies and other insects must be able to adjust to changes in light intensity over different time scales, from rapid differences during flight to gradual changes throughout the day.[88,89] It was recently shown in *D. melanogaster* that this gain control over different timescales happens downstream of the PRCs by the luminance-sensitive lamina monopolar cell (LMC) L3[88,89] (Figure 2; Box 1).

These studies in *Drosophila* have been complemented by the generation of connectomes of other insects, including the lamina of a butterfly *Papilio xuthus*[90] and the projectome of the central complex of the bumblebee *Bombus terrestris*.[91] While the acquisition and comparison of these connectomes is a complex task, it is beginning to reveal conserved and divergent aspects of visual processing.[1,90]

Chua et al. (2023)**??** recently reported their reconstruction of the adult eye structure and lamina connectome of the parasitoid wasp *Megaphragma viggianii*. Females of this remarkable insect only have 29 ommatidia in each eye and their brain only contains about 8600 cells.[1] This study verified differences in ommatidia structure in *Megaphragma* between the DRA and the rest of the eye with specialization to detect polarized light in the DRA as has been observed in other insects.[15] This specialization was also reflected in the connectivity (synapses received) of PRC R7 versus R7′ (and R8) between non-DRA and DRA, which correlated with variance in the orientation of microvilli in these regions.[1]

LMCs are key conserved laminal cell types in arthropod visual systems, which receive synapses from the PRCs and transmit information to the medulla (Figure 2; Box 1). While other hymenopterans, including *Apis*, likely only have four distinctive LMCs,[1] *Megaphragma* has five (L1–L4, LN) like *Drosophila*. Interestingly, four of the *Megaphragma* LMCs have lost their nucleus presumably as part of miniaturization.[1] Analysis of the *Megaphragma* connectome showed that R1–R6 inputs to the LMCs are mostly to L1–L3 consistent with the *Drosophila* and *Papilio* laminae (Figure 2), which suggests a similar function in detecting contrast changes in this insect.[1,90] LN in *Megaphragma* receives almost no R1–R6 synapses, which is again similar to L5 in *Drosophila* and *Papilio*.[90] Furthermore, in *Drosophila* and *Megaphragma* one LMC (L2 in *Megaphragma*) forms a large number of connections to other LMCs, for example, between L2 and L4, similar to *Drosophila* and *Apis* (Figure 2). Overall, LMC connections are generally similar between *Megaphragma*, *Drosophila*, and *Papilio* suggesting this is the ground plan for insects. However, in *Drosophila* L4 has both pre- and post-synaptic connections to the cartridge of the posterior neighbor aligned with optic flow during flight, although these connections do not appear to be involved in the detection of motion[92–95] (Figure 2B). In *Megaphragma*, the connections from L2 (in posterior cartridges) to L4 (in anterior cartridges) are only post-synaptic and opposite to optic flow (Figure 2).[1] The authors speculate that this may be associated with differences in the role of L4 between these insects.[1]

The *Megaphragma* lamina shows additional differences to other insects. For example, *Megaphragma* has no neurons homologous to Lawf, C2 and C3 neurons in *Drosophila*, which connect with the medulla.[1,95] *Megaphragma* also has far fewer lamina inter-cartridge connections compared to the lamina of *Papilio*.[1,90] The similarities and differences between the laminal connectome of *Megaphragma* to other insects show both the essential and superfluous laminal cells and connections retained and discarded respectively during miniaturization of this micro-wasp.

Taken together, these comparisons of insect connectomes also have broader implications for understanding the evolution of the neural wiring that encodes and processes visual information in these animals. First, they reveal the basic templates of cells and connections conserved across the visual processing systems of insects. Second, they highlight lineage-specific features of insect visual systems that are starting to help better understand their evolution and how this gives rise to different visual preferences and behaviors.

A complementary approach to connectomics is to genetically map natural differences in visual preferences and ask how these genes are integrated into visual processing. Rossi et al. (2024)[96] recently used such an approach to map the genetic basis for mate color preference among species of *Heliconius* butterflies. *H. melpomene* and *H. cydno* have red and white colored bands respectively on their forewings. However, *H. timarata*, the sister species of *H. cydno*, is a mimetic of *H. melpomene* that has acquired red coloration through adaptive introgression with the latter species.[97–99] *H. melpomene* and *H. timarata* males also both show mating preference for females with red patterns. The new work by Rossi et al. (2024)[96] used mapping and RNA-seq to show that *H. melpomene* and *H. timarata* preference for red patterns is associated with lower expression of *regucalcin1* compared to *H. cydno*, and that CRISPR/Cas9 mutation of this gene perturbs courtship. The identification of this role of *regucalcin1* in mating preference among *Heliconius* butterfly species now offers an excellent opportunity to better

understand how visual preferences are wired into the nervous system and how it evolves. Specifically, it will be interesting to understand the function and interactions of *regucalcin1* in the brain and where and how differences in the expression of this gene alter visual processing and color preference.

CONCLUSIONS

Our review of recent research demonstrates that the gap is closing between our detailed genetic and developmental understanding of *Drosophila* eyes and insights of visual behavior and ecology in other insects. Comparative studies linking the evolution, development, and function of visual systems across a wide range of insect species are required to gain a full understanding of the diversification of insect eyes. We propose that this would be greatly aided in the future by research focusing on four main areas.

First, the application of single cell transcriptomics in *Drosophila* has verified many of the known regulators and cell types but also identified new genes and cell markers[100–103] as well as detailed new insights into the regulation and function of the *Drosophila* visual system more broadly[104,105] (Box 2). Furthermore, combining sc-RNA-seq with other "-omics" approaches, such as single cell ATAC-seq chromatin profiling, has provided major new knowledge about the GRNs for *Drosophila* eye and optic lobe development, and predictive power to model and test the regulatory logic underling gene expression.[106–108] Applying these technologies to the developing eyes and visual systems of other insects, as recently carried out for honeybees,[109] will provide a framework to characterize how these genetic programmes evolve to produce visual systems with different morphologies, information processing, and functions. Second, overcoming challenges to culturing other insects in the lab is crucial to understand, compare, and manipulate eye development and has recently successfully been carried out for mayflies for the first time[24] (Box 2). This would facilitate RNA-seq approaches and CRISPR/Cas9, which are broadly applicable in insects to analyze gene expression and function. Third, the growing number of insect visual system connectomes has greatly advanced our understanding of the detection and processing of visual cues and how this underlies behaviors. However, higher throughput connectomics is needed to better understand the function and evolution of visual systems within and between species. Finally, even where eye development and structure are well understood, especially within and among *Drosophila* species, a greater focus on the consequences for vision and how this underpins behavioral and ecological differences as exemplified in recent studies[45,68] is needed in future research.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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REFERENCES

1. Chua, N. J., Makarova, A. A., Gunn, P., Villani, S., Cohen, B., Thasin, M., Wu, J., Shefter, D., Pang, S., Xu, C. S., Hess, H. F., Polilov, A. A., & Chklovskii, D. B. (2023). A complete reconstruction of the early visual system of an adult insect. *Current Biology*, *33*(21), 4611–4623.e4. https://doi.org/10.1016/j.cub.2023.09.021
2. Sherk, T. E. (1978). Development of the compound eyes of dragonflies (Odonata). III. Adult compound eyes. *Journal of Experimental Zoology*, *203*(1), 61–79. https://doi.org/10.1002/jez.1402030107
3. Gaspar, P., Arif, S., Sumner-Rooney, L., Kittelmann, M., Bodey, A. J., Stern, D. L., Nunes, M. D. S., & McGregor, A. P. (2020). Characterization of the genetic architecture underlying eye size variation within *Drosophila melanogaster* and *Drosophila simulans*. *G3 Genes|Genomes|Genetics*, *10*(3), 1005–1018. https://doi.org/10.1534/g3.119.400877
4. Arif, S., Hilbrant, M., Hopfen, C., Almudi, I., Nunes, M. D. S., Posnien, N., Kuncheria, L., Tanaka, K., Mitteroecker, P., Schlötterer, C., & Mcgregor, A. P. (2013). Genetic and developmental analysis of differences in eye and face morphology between *Drosophila simulans* and *Drosophila mauritiana*. *Evolution & Development*, *15*(4), 257–267. https://doi.org/10.1111/ede.12027
5. Buffry, A. D., Currea, J. P., Franke, F. A., Palavalli-Nettimi, R., Bodey, A. P., Rau, C., Samadi, N., Gstöhl, S. J., Schlepütz, C. M., McGregor, A. P., Sumner-Rooney, L., Theobald, J., & Kittelmann, M. (2024). Evolution of compound eye morphology underlies differences in vision between closely related *Drosophila* species. *BMC Biology*, *22*, 67. https://doi.org/10.1186/s12915-024-01864-7
6. Perl, C. D., & Niven, J. E. (2016). Differential scaling within an insect compound eye. *Biology Letters*, *12*(3), 20160042. https://doi.org/10.1098/rsbl.2016.0042
7. Gonzalez-Bellido, P. T., Wardill, T. J., & Juusola, M. (2011). Compound eyes and retinal information processing in miniature dipteran species match their specific ecological demands. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(10), 4224–4229. https://doi.org/10.1073/pnas.1014438108
8. Sukontason, K. L., Chaiwong, T., Piangjai, S., Upakut, S., Moophayak, K., & Sukontason, K. (2008). Ommatidia of blow fly, house fly, and flesh fly: Implication of their vision efficiency. *Parasitology Research*, *103*(1), 123–131. https://doi.org/10.1007/s00436-008-0939-y
9. Heras, F. J. H., & Laughlin, S. B. (2024). Investments in photoreceptors compete with investments in optics to determine eye design. *BioRxiv*, 2024.2001.2021.576511. https://doi.org/10.1101/2024.01.21.576511
10. Labhart, T., & Nilsson, D. E. (1995). The dorsal eye of the dragonfly *Sympetrum*—specializations for prey detection against the blue sky. *Journal of Comparative Physiology A – Neuroethology Sensory Neural and Behavioral Physiology*, *176*(4), 437–453.
11. Land, M. F., & Nilsson, D. E. (2012). *Animal eyes* (2nd ed., Oxford University Press, pp. 1–271).
12. Land, M. F. (1997). Visual acuity in insects. *Annual Review of Entomology*, *42*(1), 147–177. https://doi.org/10.1146/annurev.ento.42.1.147
13. O'carroll, D. C., & Wiederman, S. D. (2014). Contrast sensitivity and the detection of moving patterns and features. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *369*(1636), 20130043. https://doi.org/10.1098/rstb.2013.0043

14. Behnia, R., & Desplan, C. (2015). Visual circuits in flies: Beginning to see the whole picture. *Current Opinion in Neurobiology*, *34*, 125–132. https://doi.org/10.1016/j.conb.2015.03.010
15. Wernet, M. F., Perry, M. W., & Desplan, C. (2015). The evolutionary diversity of insect retinal mosaics: Common design principles and emerging molecular logic. *Trends in Genetics*, *31*(6), 316–328. https://doi.org/10.1016/j.tig.2015.04.006
16. Liénard, M. A., Bernard, G. D., Allen, A., Lassance, J.-M., Song, S., Childers, R. R., Yu, N., Ye, D., Stephenson, A., Valencia-Montoya, W. A., Salzman, S., Whitaker, M. R. L., Calonje, M., Zhang, F., & Pierce, N. E. (2021). The evolution of red color vision is linked to coordinated rhodopsin tuning in lycaenid butterflies. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(6). https://doi.org/10.1073/pnas.2008986118
17. Mcculloch, K. J., Macias-Muñoz, A., & Briscoe, A. D. (2022). Insect opsins and evo-devo: What have we learned in 25 years? *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *377*(1862), 20210288. https://doi.org/10.1098/rstb.2021.0288
18. Mulhair, P. O., Crowley, L., Boyes, D. H., Lewis, O. T., & Holland, P. W. H. (2023). Opsin gene duplication in Lepidoptera: Retrotransposition, sex linkage, and gene expression. *Molecular Biology and Evolution*, *40*(11). https://doi.org/10.1093/molbev/msad241
19. Chakraborty, M., Lara, A. G., Dang, A., Mcculloch, K. J., Rainbow, D., Carter, D., Ngo, L. T., Solares, E., Said, I., Corbett-Detig, R. B., Gilbert, L. E., Emerson, J. J., & Briscoe, A. D. (2023). Sex-linked gene traffic underlies the acquisition of sexually dimorphic UV color vision in *Heliconius* butterflies. *Proceedings of the National Academy of Sciences of the United States of America*, *120*(33), e2301411120. https://doi.org/10.1073/pnas.2301411120
20. Perry, M., Kinoshita, M., Saldi, G., Huo, L., Arikawa, K., & Desplan, C. (2016). Molecular logic behind the three-way stochastic choices that expand butterfly colour vision. *Nature*, *535*(7611), 280–284. https://doi.org/10.1038/nature18616
21. Johnston, R. J., Otake, Y., Sood, P., Vogt, N., Behnia, R., Vasiliauskas, D., Mcdonald, E., Xie, B., Koenig, S., Wolf, R., Cook, T., Gebelein, B., Kussell, E., Nakagoshi, H., & Desplan, C. (2011). Interlocked feedforward loops control cell-type-specific Rhodopsin expression in the *Drosophila* eye. *Cell*, *145*(6), 956–968. https://doi.org/10.1016/j.cell.2011.05.003
22. Agi, E., Langen, M., Altschuler, S. J., Wu, L. F., Zimmermann, T., & Hiesinger, P. R. (2014). The evolution and development of neural superposition. *Journal of Neurogenetics*, *28*(3-4), 216–232. https://doi.org/10.3109/01677063.2014.922557
23. Warrant, E. J. (2017). The remarkable visual capacities of nocturnal insects: Vision at the limits with small eyes and tiny brains. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *20160063*(1717), 20160063. https://doi.org/10.1098/rstb.2016.0063
24. Almudi, I., Martín-Blanco, C. A., García-Fernandez, I. M., López-Catalina, A., Davie, K., Aerts, S., & Casares, F. (2019). Establishment of the mayfly *Cloeon dipterum* as a new model system to investigate insect evolution. *EvoDevo*, *10*, 6. https://doi.org/10.1186/s13227-019-0120-y
25. Almudi, I., Vizueta, J., Wyatt, C. D. R., De Mendoza, A., Marlétaz, F., Firbas, P. N., Feuda, R., Masiero, G., Medina, P., Alcaina-Caro, A., Cruz, F., Gómez-Garrido, J., Gut, M., Alioto, T. S., Vargas-Chavez, C., Davie, K., Misof, B., González, J., Aerts, S., … Casares, F. (2020). Genomic adaptations to aquatic and aerial life in mayflies and the origin of insect wings. *Nature Communications*, *11*(1), 2631. https://doi.org/10.1038/s41467-020-16284-8
26. Rathore, S., Meece, M., Charlton-Perkins, M., Cook, T. A., & Buschbeck, E. K. (2023). Probing the conserved roles of cut in the development and function of optically different insect compound eyes. *Frontiers in Cell and Developmental Biology*, *11*, 1104620. https://doi.org/10.3389/fcell.2023.1104620
27. Buschbeck, E. K., & Friedrich, M. (2008). Evolution of insect eyes: Tales of ancient heritage, deconstruction, reconstruction, remodeling, and recycling. *Evolution: Education and Outreach*, *1*(4), 448–462. https://doi.org/10.1007/s12052-008-0086-z
28. Warren, J., & Kumar, J. P. (2023). Patterning of the *Drosophila* retina by the morphogenetic furrow. *Frontiers in Cell and Developmental Biology*, *11*, 1151348. https://doi.org/10.3389/fcell.2023.1151348
29. Neriec, N., & Desplan, C. (2016). From the eye to the brain: Development of the *Drosophila* visual system. *Current Topics in Developmental Biology*, *116*, 247–271. https://doi.org/10.1016/bs.ctdb.2015.11.032
30. Atkins, M., & Mardon, G. (2009). Signaling in the third dimension: The peripodial epithelium in eye disc development. *Developmental Dynamics*, *238*(9), 2139–2148. https://doi.org/10.1002/dvdy.22034
31. Treisman, J. E. (2013). Retinal differentiation in *Drosophila*. *Wiley Interdisciplinary Reviews: Developmental Biology*, *2*(4), 545–557. https://doi.org/10.1002/wdev.100
32. Navarro, T., Iannini, A., Neto, M., Campoy-Lopez, A., Muñoz-García, J., Pereira, P. S., Ares, S., & Casares, F. (2024). Feedback control of organ size precision is mediated by BMP2-regulated apoptosis in the *Drosophila* eye. *PLoS Biology*, *22*(1), e3002450. https://doi.org/10.1371/journal.pbio.3002450
33. Chen, Q., Sasikala-Appukuttan, A. K., Husain, Z., Shrivastava, A., Spain, M., Sendler, E. D., Daines, B., Fischer, S., Chen, R., Cook, T. A., & Friedrich, M. (2023). Global gene expression analysis reveals complex cuticle organization of the *Tribolium* compound eye. *Genome Biology and Evolution*, *15*(1). https://doi.org/10.1093/gbe/evac181
34. Friedrich, M., Rambold, I., & Melzer, R. R. (1996). The early stages of ommatidial development in the flour beetle *Tribolium castaneum* (Coleoptera; Tenebrionidae). *Development Genes and Evolution*, *206*(2), 136–146. https://doi.org/10.1007/s004270050039
35. Marco Antonio, D. S., & Hartfelder, K. (2017). Toward an understanding of divergent compound eye development in drones and workers of the honeybee (*Apis mellifera* L.): A correlative analysis of morphology and gene expression. *Journal of Experimental Zoology Part B – Molecular and Developmental Evolution*, *328*(1-2), 139–156. https://doi.org/10.1002/jez.b.22696
36. Eisen, J. S., & Youssef, N. N. (1980). Fine structural aspects of the developing compound eye of the honey bee, *Apis mellifera* L. *Journal of Ultrastructure Research*, *71*(1), 79–94. https://doi.org/10.1016/s0022-5320(80)90038-6
37. Friedrich, M. (2006). Continuity versus split and reconstitution: Exploring the molecular developmental corollaries of insect eye primordium evolution. *Developmental Biology*, *299*(2), 310–329. https://doi.org/10.1016/j.ydbio.2006.08.027
38. Casares, F., & McGregor, A. P. (2021). The evolution and development of eye size in flies. *Wiley Interdisciplinary Reviews: Developmental Biology*, *10*(2), e380. https://doi.org/10.1002/wdev.380
39. Chen, Y. C., & Desplan, C. (2020). Gene regulatory networks during the development of the *Drosophila* visual system. *Current Topics in Developmental Biology*, *139*, 89–125. https://doi.org/10.1016/bs.ctdb.2020.02.010
40. Koenig, K. M., & Gross, J. M. (2020). Evolution and development of complex eyes: A celebration of diversity. *Development (Cambridge, England)*, *147*(19). https://doi.org/10.1242/dev.182923
41. Mahato, S., Morita, S., Tucker, A. E., Liang, X., Jackowska, M., Friedrich, M., Shiga, Y., & Zelhof, A. C. (2014). Common transcriptional mechanisms for visual photoreceptor cell differentiation among Pancrustaceans. *PLoS Genetics*, *10*(7), e1004484. https://doi.org/10.1371/journal.pgen.1004484
42. Zarinkamar, N., Yang, X., Bao, R., Friedrich, F., Beutel, R., & Friedrich, M. (2011). The Pax gene eyegone facilitates repression of eye development in *Tribolium*. *EvoDevo*, *2*(1), 8. https://doi.org/10.1186/2041-9139-2-8
43. Keesey, I. W., Grabe, V., Gruber, L., Koerte, S., Obiero, G. F., Bolton, G., Khallaf, M. A., Kunert, G., Lavista-Llanos, S., Valenzano, D. R.,

Rybak, J., Barrett, B. A., Knaden, M., & Hansson, B. S. (2019). Inverse resource allocation between vision and olfaction across the genus *Drosophila*. *Nature Communications*, *10*(1), 1162. https://doi.org/10.1038/s41467-019-09087-z

44. Posnien, N., Hopfen, C., Hilbrant, M., Ramos-Womack, M., Murat, S., Schönauer, A., Herbert, S. L., Nunes, M. D. S., Arif, S., Breuker, C. J., Schlötterer, C., Mitteroecker, P., & Mcgregor, A. P. (2012). Evolution of eye morphology and rhodopsin expression in the *Drosophila melanogaster* species subgroup. *PLoS ONE*, *7*(5), e37346. https://doi.org/10.1371/journal.pone.0037346
45. Keesey, I. W., Grabe, V., Knaden, M., & Hansson, B. S. (2020). Divergent sensory investment mirrors potential speciation via niche partitioning across *Drosophila*. *eLife*, *9*, https://doi.org/10.7554/eLife.57008
46. Hilbrant, M., Almudi, I., Leite, D. J., Kuncheria, L., Posnien, N., Nunes, M. D., & Mcgregor, A. P. (2014). Sexual dimorphism and natural variation within and among species in the *Drosophila* retinal mosaic. *BMC Evolutionary Biology*, *14*, 240. https://doi.org/10.1186/s12862-014-0240-x
47. Ramaekers, A., Claeys, A., Kapun, M., Mouchel-Vielh, E., Potier, D., Weinberger, S., Grillenzoni, N., Dardalhon-Cuménal, D., Yan, J., Wolf, R., Flatt, T., Buchner, E., & Hassan, B. A. (2019). Altering the temporal regulation of one transcription factor drives evolutionary trade-offs between head sensory organs. *Developmental Cell*, *50*(6), 780–792.e7. https://doi.org/10.1016/j.devcel.2019.07.027
48. Reis, M., Wiegleb, G., Claude, J., Lata, R., Horchler, B., Ha, N.-T., Reimer, C., Vieira, C. P., Vieira, J., & Posnien, N. (2020). Multiple loci linked to inversions are associated with eye size variation in species of the *Drosophila virilis* phylad. *Scientific Reports*, *10*(1), 12832. https://doi.org/10.1038/s41598-020-69719-z
49. Norry, F. M., & Gomez, F. H. (2017). Quantitative trait loci and antagonistic associations for two developmentally related traits in the *Drosophila* head. *Journal of Insect Science (Online)*, *17*(1), 19. https://doi.org/10.1093/jisesa/iew115
50. Buchberger, E., Bilen, A., Ayaz, S., Salamanca, D., Matas De Las Heras, C., Niksic, A., Almudi, I., Torres-Oliva, M., Casares, F., & Posnien, N. (2021). Variation in pleiotropic hub gene expression is associated with interspecific differences in head shape and eye size in *Drosophila*. *Molecular Biology and Evolution*, *38*(5), 1924–1942. https://doi.org/10.1093/molbev/msaa335
51. Weinkove, D., Neufeld, T. P., Twardzik, T., Waterfield, M. D., & Leevers, S. J. (1999). Regulation of imaginal disc cell size, cell number and organ size by *Drosophila* class I(A) phosphoinositide 3-kinase and its adaptor. *Current Biology*, *9*(18), 1019–1029. https://doi.org/10.1016/s0960-9822(99)80450-3
52. Montserrat, T.-O., Elisa, B., Alexandra, D. B., Maike, K., Lauren, S.-R., Pedro, G., Bullinger, G. C., Guerrero, G., Casares, F., Arif, S., Posnien, N., Nunes, M. D. S., McGregor, A. P., & Isabel, A. (2021). Differences in *orthodenticle* expression promote ommatidial size variation between *Drosophila* species. *BioRxiv*, 2021.2003.2017.435774. https://doi.org/10.1101/2021.03.17.435774
53. Netschitailo, O., Wang, Y., Wagner, A., Sommer, V., Verhulst, E. C., & Beye, M. (2023). The function and evolution of a genetic switch controlling sexually dimorphic eye differentiation in honeybees. *Nature Communications*, *14*(1), 463. https://doi.org/10.1038/s41467-023-36153-4
54. Menzel, J. G., Wunderer, H., & Stavenga, D. G. (1991). Functional morphology of the divided compound eye of the honeybee drone (*Apis mellifera*). *Tissue & Cell*, *23*(4), 525–535. https://doi.org/10.1016/0040-8166(91)90010-q
55. Van Praagh, J. P., Ribi, W., Wehrhahn, C., & Wittmann, D. (1980). Drone bees fixate the queen with the dorsal frontal part of their compound eyes. *Journal of Comparative Physiology*, *136*(3), 263–266. https://doi.org/10.1007/BF00657542
56. Streinzer, M., Brockmann, A., Nagaraja, N., & Spaethe, J. (2013). Sex and caste-specific variation in compound eye morphology of five honeybee species. *PLoS ONE*, *8*(2), e57702. https://doi.org/10.1371/journal.pone.0057702
57. Egelhaaf, M., & Kern, R. (2002). Vision in flying insects. *Current Opinion in Neurobiology*, *12*(6), 699–706. https://doi.org/10.1016/s0959-4388(02)00390-2
58. Gonzalez-Bellido, P. T., Talley, J., & Buschbeck, E. K. (2022). Evolution of visual system specialization in predatory arthropods. *Current Opinion in Insect Science*, *52*, 100914. https://doi.org/10.1016/j.cois.2022.100914
59. Meece, M., Rathore, S., & Buschbeck, E. K. (2021). Stark trade-offs and elegant solutions in arthropod visual systems. *Journal of Experimental Biology*, *224*(Pt 4). https://doi.org/10.1242/jeb.215541
60. Warrant, E., & Somanathan, H. (2022). Colour vision in nocturnal insects. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *377*(1862), 20210285. https://doi.org/10.1098/rstb.2021.0285
61. Song, B.-M., & Lee, C.-H. (2018). Toward a mechanistic understanding of color vision in insects. *Frontiers in Neural Circuits*, *12*, 16. https://doi.org/10.3389/fncir.2018.00016
62. Kinoshita, M., & Arikawa, K. (2023). 'Color' processing in the butterfly visual system. *Trends in Neuroscience (Tins)*, *46*(5), 338–340. https://doi.org/10.1016/j.tins.2023.02.006
63. Lancer, B. H., Evans, B. J. E., & Wiederman, S. D. (2020). The visual neuroecology of anisoptera. *Current Opinion in Insect Science*, *42*, 14–22. https://doi.org/10.1016/j.cois.2020.07.002
64. Supple, J. A., Pinto-Benito, D., Khoo, C., Wardill, T. J., Fabian, S. T., Liu, M., Pusdekar, S., Galeano, D., Pan, J., Jiang, S., Wang, Y., Liu, L., Peng, H., Olberg, R. M., & Gonzalez-Bellido, P. T. (2020). Binocular encoding in the damselfly pre-motor target tracking system. *Current Biology*, *30*(4), 645–656.e4. https://doi.org/10.1016/j.cub.2019.12.031
65. Vijayan, S., Balamurali, G. S., Johnson, J., Kelber, A., Warrant, E. J., & Somanathan, H. (2023). Dim-light colour vision in the facultatively nocturnal Asian giant honeybee, *Apis dorsata*. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *290*(2004), 20231267. https://doi.org/10.1098/rspb.2023.1267
66. Nunes, M. D. S., Arif, S., Schlötterer, C., & Mcgregor, A. P. (2013). A perspective on micro-evo-devo: Progress and potential. *Genetics*, *195*(3), 625–634. https://doi.org/10.1534/genetics.113.156463
67. Estrada, C., & Jiggins, C. D. (2002). Patterns of pollen feeding and habitat preference among *Heliconius* species. *Ecological Entomology*, *27*(4), 448–456. doi: https://doi.org/10.1046/j.1365-2311.2002.00434.x
68. Wright, D. S., Rodriguez-Fuentes, J., Ammer, L., Darragh, K., Kuo, C.-Y., McMillan, W. O., Jiggins, C. D., Montgomery, S. H., & Merrill, R. M. (2023). Adaptive divergence in the eyes of *Heliconius* butterflies likely contributes to pre- and post-mating isolation. *BioRxiv*, https://doi.org/10.1101/2023.10.26.564160
69. Montgomery, S. H., Rossi, M., Mcmillan, W. O., & Merrill, R. M. (2021). Neural divergence and hybrid disruption between ecologically isolated Heliconius butterflies. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(6). https://doi.org/10.1073/pnas.2015102118
70. Seymoure, B. M., Mcmillan, W. O., & Rutowski, R. (2015). Peripheral eye dimensions in Longwing (*Heliconius*) butterflies vary with body size and sex but not light environment nor mimicry ring. *Journal of Research on the Lepidoptera*, *48*, 83–92.
71. Byers, K., Darragh, K., Fernanda Garza, S., Abondano Almeida, D., Warren, I. A., Rastas, P. M. A., Merrill, R. M., Schulz, S., Mcmillan, W. O., & Jiggins, C. D. (2021). Clustering of loci controlling species differences in male chemical bouquets of sympatric *Heliconius* butterflies. *Ecology & Evolution*, *11*(1), 89–107. https://doi.org/10.1002/ece3.6947

72. Taylor, G. J., Tichit, P., Schmidt, M. D., Bodey, A. J., Rau, C., & Baird, E. (2019). Bumblebee visual allometry results in locally improved resolution and globally improved sensitivity. *eLife*, *8*, https://doi.org/10.7554/eLife.40613
73. Bartholomée, O., Dwyer, C., Tichit, P., Caplat, P., Baird, E., & Smith, H. G. (2023). Shining a light on species coexistence: Visual traits drive bumblebee communities. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *290*(1996), 20222548. https://doi.org/10.1098/rspb.2022.2548
74. Stöckl, A., Grittner, R., Taylor, G., Rau, C., Bodey, A. J., Kelber, A., & Baird, E. (2022). Allometric scaling of a superposition eye optimizes sensitivity and acuity in large and small hawkmoths. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *289*(1979), 20220758. https://doi.org/10.1098/rspb.2022.0758
75. Land, M. F. (1999). Motion and vision: Why animals move their eyes. *Journal of Comparative Physiology A*, *185*(4), 341–352. https://doi.org/10.1007/s003590050393
76. Cellini, B., & Mongeau, J.-M. (2020). Active vision shapes and coordinates flight motor responses in flies. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(37), 23085–23095. https://doi.org/10.1073/pnas.1920846117
77. Ravi, S., Siesenop, T., Bertrand, O. J., Li, L., Doussot, C., Fisher, A., Warren, W. H., & Egelhaaf, M. (2022). Bumblebees display characteristics of active vision during robust obstacle avoidance flight. *Journal of Experimental Biology*, *225*(4). https://doi.org/10.1242/jeb.243021
78. Burtt, E. T., & Patterson, J. A. (1970). Internal muscle in the eye of an insect. *Nature*, *228*(5267), 183–184. https://doi.org/10.1038/228183a0
79. Fenk, L. M., Avritzer, S. C., Weisman, J. L., Nair, A., Randt, L. D., Mohren, T. L., Siwanowicz, I., & Maimon, G. (2022). Muscles that move the retina augment compound eye vision in *Drosophila*. *Nature*, *612*(7938), 116–122. https://doi.org/10.1038/s41586-022-05317-5
80. Kemppainen, J., Scales, B., Razban Haghighi, K., Takalo, J., Mansour, N., Mcmanus, J., Leko, G., Saari, P., Hurcomb, J., Antohi, A., Suuronen, J.-P., Blanchard, F., Hardie, R. C., Song, Z., Hampton, M., Eckermann, M., Westermeier, F., Frohn, J., Hoekstra, H., … Juusola, M. (2022). Binocular mirror-symmetric microsaccadic sampling enables *Drosophila* hyperacute 3D vision. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(12), e2109717119. https://doi.org/10.1073/pnas.2109717119
81. Hardie, R. C., & Franze, K. (2012). Photomechanical responses in *Drosophila* photoreceptors. *Science*, *338*(6104), 260–263. https://doi.org/10.1126/science.1222376
82. Kemppainen, J., Mansour, N., Takalo, J., & Juusola, M. (2022). High-speed imaging of light-induced photoreceptor microsaccades in compound eyes. *Communications Biology*, *5*(1), 203. https://doi.org/10.1038/s42003-022-03142-0
83. Shinomiya, K., Nern, A., Meinertzhagen, I. A., Plaza, S. M., & Reiser, M. B. (2022). Neuronal circuits integrating visual motion information in *Drosophila melanogaster*. *Current Biology*, *32*(16), 3529–3544.e2. https://doi.org/10.1016/j.cub.2022.06.061
84. Schnaitmann, C., Haikala, V., Abraham, E., Oberhauser, V., Thestrup, T., Griesbeck, O., & Reiff, D. F. (2018). Color processing in the early visual system of *Drosophila*. *Cell*, *172*(1-2), 318–330.e18. https://doi.org/10.1016/j.cell.2017.12.018
85. Pagni, M., Haikala, V., Oberhauser, V., Meyer, P. B., Reiff, D. F., & Schnaitmann, C. (2021). Interaction of "chromatic" and "achromatic" circuits in *Drosophila* color opponent processing. *Current Biology*, *31*(8), 1687–1698.e4. https://doi.org/10.1016/j.cub.2021.01.105
86. Li, Y., Chen, P.-J., Lin, T.-Y., Ting, C.-Y., Muthuirulan, P., Pursley, R., Ilić, M., Pirih, P., Drews, M. S., Menon, K. P., Zinn, K. G., Pohida, T., Borst, A., & Lee, C.-H. (2021). Neural mechanism of spatio-chromatic opponency in the *Drosophila* amacrine neurons. *Current Biology*, *31*(14), 3040–3052.e9. https://doi.org/10.1016/j.cub.2021.04.068
87. Dustin, G., Emil, K., Aljoscha, N., Lucy, H., Arthur, Z., Gizem, S., Rubin, G. M., Wernet, M. F., & Sung Soo, K. (2023). Connectomic reconstruction predicts the functional organization of visual inputs to the navigation center of the *Drosophila* brain. *bBioRxiv*, 2023.2011.2029.569241. https://doi.org/10.1101/2023.11.29.569241
88. Ketkar, M. D., Gür, B., Molina-Obando, S., Ioannidou, M., Martelli, C., & Silies, M. (2022). First-order visual interneurons distribute distinct contrast and luminance information across ON and OFF pathways to achieve stable behavior. *eLife*, *11*, https://doi.org/10.7554/eLife.74937
89. Ketkar, M. D., Shao, S., Gjorgjieva, J., & Silies, M. (2023). Multifaceted luminance gain control beyond photoreceptors in *Drosophila*. *Current Biology*, *33*(13), 2632–2645.e6. https://doi.org/10.1016/j.cub.2023.05.024
90. Matsushita, A., Stewart, F., Ilić, M., Chen, P.-J., Wakita, D., Miyazaki, N., Murata, K., Kinoshita, M., Belušič, G., & Arikawa, K. (2022). Connectome of the lamina reveals the circuit for early color processing in the visual pathway of a butterfly. *Current Biology*, *32*(10), 2291–2299.e3. https://doi.org/10.1016/j.cub.2022.03.066
91. Sayre, M. E., Templin, R., Chavez, J., Kempenaers, J., & Heinze, S. (2021). A projectome of the bumblebee central complex. *eLife*, *10*, https://doi.org/10.7554/eLife.68911
92. Silies, M., Gohl, D. M., Fisher, Y. E., Freifeld, L., Clark, D. A., & Clandinin, T. R. (2013). Modular use of peripheral input channels tunes motion-detecting circuitry. *Neuron*, *79*(1), 111–127. https://doi.org/10.1016/j.neuron.2013.04.029
93. Meinertzhagen, I. A., & O'neil, S. D. (1991). Synaptic organization of columnar elements in the lamina of the wild type in *Drosophila melanogaster*. *Journal of Comparative Neurology*, *305*(2), 232–263. https://doi.org/10.1002/cne.903050206
94. Rivera-Alba, M., Vitaladevuni, S. N., Mishchenko, Y., Lu, Z., Takemura, S.-Y., Scheffer, L., Meinertzhagen, I. A., Chklovskii, D. B., & De Polavieja, G. G. (2011). Wiring economy and volume exclusion determine neuronal placement in the *Drosophila* brain. *Current Biology*, *21*(23), 2000–2005. https://doi.org/10.1016/j.cub.2011.10.022
95. Tuthill, J. C., Nern, A., Holtz, S. L., Rubin, G. M., & Reiser, M. B. (2013). Contributions of the 12 neuron classes in the fly lamina to motion vision. *Neuron*, *79*(1), 128–140. https://doi.org/10.1016/j.neuron.2013.05.024
96. Rossi, M., Hausmann, A. E., Alcami, P., Moest, M., Wright, D. S., Kuo, C-Y., Lozano-Urrego, D., Maulana, A., Melo-Flórez, L., Rueda-Munoz, G., McMahon, S., Linares, M., Osman, C., McMillan, W. O., Pardo-Diaz, C., Salazar, C., & Merrill, R. M. (2024). Adaptive introgression of a visual preference gene. *Science*, *383*(6689), 1368–1373. https://doi.org/10.1126/science.adj9201
97. Wallbank, R. W. R., Baxter, S. W., Pardo-Diaz, C., Hanly, J. J., Martin, S. H., Mallet, J., Dasmahapatra, K. K., Salazar, C., Joron, M., Nadeau, N., Mcmillan, W. O., & Jiggins, C. D. (2016). Evolutionary novelty in a butterfly wingpattern through enhancer shuffling. *PLoS Biology*, *14*(1), e1002353. https://doi.org/10.1371/journal.pbio.1002353
98. *Heliconius* Genome Consortium. (2012). Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature*, *487*(7405), 94–98. https://doi.org/10.1038/nature11041
99. Pardo-Diaz, C., Salazar, C., Baxter, S. W., Merot, C., Figueiredo-Ready, W., Joron, M., Mcmillan, W. O., & Jiggins, C. D. (2012). Adaptive introgression across species boundaries in *Heliconius* butterflies. *PLoS Genetics*, *8*(6), e1002752. https://doi.org/10.1371/journal.pgen.1002752
100. Ariss, M. M., Islam, A., Critcher, M., Zappia, M. P., & Frolov, M. V. (2018). Single cell RNA-sequencing identifies a metabolic aspect of apoptosis in Rbf mutant. *Nature Communications*, *9*(1), 5024. https://doi.org/10.1038/s41467-018-07540-z
101. Rader, A. E., Bayarmagnai, B., & Frolov, M. V. (2023). Combined inactivation of RB and Hippo converts differentiating *Drosophila*

photoreceptors into eye progenitor cells through derepression of *homothorax*. *Developmental Cell*, *58*(21), 2261–2274.e6. https://doi.org/10.1016/j.devcel.2023.09.003

102. Wiegleb, G., Reinhardt, S., Dahl, A., & Posnien, N. (2022). Tissue dissociation for single-cell and single-nuclei RNA sequencing for low amounts of input material. *Frontiers in Zoology*, *19*(1), 27. https://doi.org/10.1186/s12983-022-00472-x
103. Yeung, K., Bollepogu Raja, K. K., Shim, Y.-K., Li, Y., Chen, R., & Mardon, G. (2022). Single cell RNA sequencing of the adult *Drosophila* eye reveals distinct clusters and novel marker genes for all major cell types. *Communications Biology*, *5*(1), 1370. https://doi.org/10.1038/s42003-022-04337-1
104. Konstantinides, N., Holguera, I., Rossi, A. M., Escobar, A., Dudragne, L., Chen, Y.-C., Tran, T. N., Martínez Jaimes, A. M., Özel, M. N., Simon, F., Shao, Z., Tsankova, N. M., Fullard, J. F., Walldorf, U., Roussos, P., & Desplan, C. (2022). A complete temporal transcription factor series in the fly visual system. *Nature*, *604*(7905), 316–322. https://doi.org/10.1038/s41586-022-04564-w
105. Ferreira, A. A. G., & Desplan, C. (2023). An atlas of the developing *Drosophila* visual aystem glia and subcellular mRNA localization of transcripts in single cells. *BioRxiv*, https://doi.org/10.1101/2023.08.06.552169
106. Bravo González-Blas, C., Quan, X.-J., Duran-Romaña, R., Taskiran, I. I., Koldere, D., Davie, K., Christiaens, V., Makhzami, S., Hulselmans, G., De Waegeneer, M., Mauduit, D., Poovathingal, S., Aibar, S., & Aerts, S. (2020). Identification of genomic enhancers through spatial integration of single-cell transcriptomics and epigenomics. *Molecular Systems Biology*, *16*(5), e9438. https://doi.org/10.15252/msb.20209438
107. Özel, M. N., Gibbs, C. S., Holguera, I., Soliman, M., Bonneau, R., & Desplan, C. (2022). Coordinated control of neuronal differentiation and wiring by sustained transcription factors. *Science*, *378*(6626), eadd1884. https://doi.org/10.1126/science.add1884
108. Bollepogu Raja, K. K., Yeung, K., Shim, Y.-K., Li, Y., Chen, R., & Mardon, G. (2023). A single cell genomics atlas of the *Drosophila* larval eye reveals distinct photoreceptor developmental timelines. *Nature Communications*, *14*(1), 7205. https://doi.org/10.1038/s41467-023-43037-0
109. Patir, A., Raper, A., Fleming, R., Henderson, B. E. P., Murphy, L., Henderson, N. C., Clark, E. L., Freeman, T. C., & Barnett, M. W. (2023). Cellular heterogeneity of the developing worker honey bee (*Apis mellifera*) pupa: A single cell transcriptomics analysis. *G3 Genes|Genomes|Genetics*, *13*(10), jkad178. https://doi.org/10.1093/g3journal/jkad178

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