Phylogenomics of opsin genes in Diptera reveals lineage-specific events and contrasting evolutionary dynamics in Anopheles and Drosophila.

Roberto Feuda\textsuperscript{a,b)*, Matthew Goulty\textsuperscript{a}, Nicola Zadra\textsuperscript{c,d}, Tiziana Gasparetti\textsuperscript{d}, Ezio Rosato\textsuperscript{a}, Davide Pisani\textsuperscript{a}, Annapaola Rizzoli\textsuperscript{c}, Nicola Segata\textsuperscript{d}, Lino Ometto\textsuperscript{f}, Omar Rota Stabelli\textsuperscript{c,g)*

\textsuperscript{a}Department of Genetics and Genome Biology, University of Leicester, UK
\textsuperscript{b}Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Naples, Italy
\textsuperscript{c}Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all’Adige, Italy
\textsuperscript{d}Department CIBIO, University of Trento, Trento, Italy
\textsuperscript{e}School of Earth Sciences, University of Bristol, UK
\textsuperscript{f}Department of Biology and Biotechnology, University of Pavia, 27100 Pavia, Italy
\textsuperscript{g}Center Agriculture Food Environment (C3A), University of Trento, San Michele all’Adige, Italy

*Authors for correspondence: Roberto Feuda rf190@leicester.ac.uk and Omar Rota-Stabelli omar.rotastabelli@unitn.it

© The Author(s) 2021. Published by Oxford University Press on behalf of the Society for Molecular Biology and Evolution. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
Significance
Diptera is an insect order including flies, mosquitoes, and various other species of economic importance. Their vision is mediated by the opsin genes, which have been studied in a few key model species. However, a comprehensive comparative genomic analysis does not exist, impairing our understanding of the evolutionary history of these genes in this order. In this work, we perform the first genome-scale analysis of opsin gene evolution in Diptera. We investigate their pattern of duplication, selection and expression in more than 60 species that belong to 10 different families. Our results clarify the evolution of the opsin genes in dipterans, in particular in fruit flies and mosquitoes, and represent the foundation for functional studies on their visual system.
Abstract

Diptera is one of the biggest insect orders and displays a large diversity of visual adaptations. Similarly to other animals, the dipteran visual process is mediated by opsin genes. While the diversity and function of these genes is well studied in key model species, a comprehensive comparative genomic study across the dipteran phylogeny is missing. Here we mined the genomes of 61 dipteran species, reconstructed the evolutionary affinities of 528 opsin genes and determined the selective pressure acting in different species. We found that opsins underwent several lineage-specific events, including an independent expansion of Long Wave Sensitive opsins in flies and mosquitoes, and numerous family-specific duplications and losses. Both the *Drosophila* and the *Anopheles* complement is derived in comparison with the ancestral dipteran state. Molecular evolutionary studies suggest that gene turnover rate, overall mutation rate, and site-specific selective pressure are higher in *Anopheles* than in *Drosophila*. Overall, our findings indicate an extremely variable pattern of opsin evolution in dipters, showcasing how two similarly aged radiations, *Anopheles* and *Drosophila*, are characterized by contrasting dynamics in the evolution of this gene family. These results provide a foundation for future studies on the dipteran visual system.
Introduction

The ability to detect and respond to specific visual stimuli and light conditions is fundamental in defining animal biology and ecology, including mating, and predatory and foraging behaviour (Tierney et al. 2015, 2011; Futahashi et al. 2015; Feuda et al. 2016; van der Kooi et al. 2021). In all animals, visual processing is mediated by opsins, a group of photosensitive G-protein coupled receptors, which originated in pre-bilatera metazoans by an ancient duplication from non-light-sensitive receptors; subsequent duplications generated C-opsins, R-opsins and Go-opsins (Feuda et al. 2012; Ramirez et al. 2016). Opsins are generally expressed in photoreceptor cells, where they mediate light sensing (Fain et al. 2010). The modification of opsin complement (such as gene duplication or loss) and/or specific functional amino acid mutations in opsin genes can confer the ability to adapt to new ecological niches, for example by providing the ability to respond to different wavelengths of light (Feuda et al. 2016; Tierney et al. 2015, 2011; Sondhi et al. 2020; see van der Kooi et al. 2021 for a recent review). However, increasing evidence indicates that, at least in the model organism *Drosophila melanogaster*, the function of the opsins is not restricted to photoreceptor cells but extends to different sensory modalities, such as mechanosensation (Zanini et al. 2018), taste (Leung et al. 2020), temperature sensation (Sokabe et al. 2016), and circadian clocks (Ni et al. 2017).

Diptera is an insect order containing more than 125,000 species (Skevington & Dang 2002), representing approximately 10% of animal diversity. This order comprises *Drosophila* and several species of economic importance, such as agricultural pests (e.g., fruit flies of genera *Bactrocera* and *Ceratitis*, as well as *Drosophila suzukii*) and vectors of infectious disease (e.g., *Glossina* tsetse flies and mosquitoes of the *Aedes*, *Anopheles* and *Culex* genera) (White & Elson-Harris 1992; Attardo et al. 2014, 2019; Neafsey et al. 2015; Rota-Stabelli et al. 2020; Zadra et al. 2021). Dipterans are characterised by a great variety of morphological, physiological and ecological behaviours resulting from a rapid radiation (Wiegmann et al. 2011). Dipterans are also characterised by a large variation in sensitivity to light (van der Kooi et al. 2021). Even within the same genus, it is possible to observe diurnal, nocturnal and crepuscular species (Table S1 and references therein).

In Diptera, and insects in general, the visual process is mediated by R-opsins which are classified according to the wavelength at which they show maximum absorbance: Long-Wavelength Sensitive opsins (LWS, sometimes known as LW) can respond to green light, Short Wavelength Sensitive opsins (SWS, sometimes known as SW) to blue light, UV opsins to ultraviolet light, and Rh7 opsins to a broad spectrum of light (Feuda et al. 2016; Sakai et al. 2017; Ni et al. 2017; van der Kooi et al. 2021; Briscoe & Chittka 2001; Henze & Oakley 2015; Fleming et al. 2018). In *Drosophila melanogaster*, the opsins are well characterized and seven genes/proteins have been identified: Rh5 respond to blue light (SWS), Rh1, Rh2 and Rh6 to green (LWS) light and Rh3 and Rh4 to UV light, (Sakai et al. 2017; Carulli et al. 1994; Bao & Friedrich 2009). The absorbance of Rh7 is particularly broad with a maximum in the UV light, but with a long tail encompassing the blue and cyan wavelengths (Sakai et al., 2017). Rh7 is expressed in a limited number of neurons in the central brain, including some of those responsible for circadian activity (Ni et al. 2017 and Ma et al. 2021). Rh2 is expressed in the ocelli (Pollock & Benzer 1989) and possibly in the R7 photoreceptor cells. All other opsins are expressed mainly in retinal photoreceptor cells which in different combination are used to define the visual competence of different photoreceptor subtypes (Nérie & Desplan 2016). However, it is not known if the opsin repertoire is conserved throughout the genus *Drosophila*.
The opsin complement has been characterised in some other dipterans, such as in various *Glossina* species (Attardo et al. 2019), in *Lucilia cuprina* (Anstead et al. 2016), and in *Calliphora vicina* (Schmitt et al. 2005) where a similar opsin complement has been indentified (Rh1, Rh2, Rh3, Rh5 and Rh6). A recent analysis performed by Giraldo-Calderón and collaborators (Giraldo-Calderón et al. 2017) on three species of Culicidae, i.e. *Aedes aegypti, Culex quinquefasciatus* and *Anopheles gambiae* identified a series of duplication events affecting LWS-Rh6 in this clade. However, whether these duplications are shared with other Culicidae remains unclear. Furthermore, other opsin genes such as arthropsins (belonging to the R-opsins), C opsins and RGR/GO opsins have been identified in some insect groups (Almudi et al. 2020; Futahashi et al. 2015; Fleming et al. 2018). However, their function, presence and potential distribution in dipterans are ambiguous (Velarde et al. 2005).

Despite the key role played by opsins in sensory biology, we lack a systematic understanding of their evolution along the dipteran phylogeny. How many opsins were present in the last dipteran common ancestor? Do the opsins in the different groups undergo similar evolutionary patterns? A rigorous comparison of opsin content in model genus *Drosophila* and *Anopheles* has also never been undertaken, leaving open such questions as whether the opsin repertoire is conserved throughout the genus and if selective forces are acting differently in different species. To address these questions, we investigated the evolution of opsin genes in 61 dipteran species sampled from ten different families and reconstructed their pattern of gene duplication and loss. We focused on the two iconic genera, *Drosophila* and *Anopheles*, and investigated the expression and occurrence of positive selection acting on the different opsin genes. Overall, our comparative genomics investigation provides an updated overview on the pattern of duplication and loss, as well as evidence for lineage-specific evolutionary histories of opsin genes in Diptera, and provides a foundation for future functional studies on the dipteran visual system.

**RESULTS AND DISCUSSION**

**Dipterans have at least eight paralogous opsin groups**

We based our analyses on the genomic data of 61 species belonging to ten different families of dipterans (Table S1). Overall, the proteome completeness values estimated by BUSCO ranged from 68.5% in *Phlebotomus papatasi* to 99.93% in *Drosophila melanogaster* (Table S1). To identify the opsin genes, we used a combination of BLAST, motif search and manual curation to minimize the possibility of false negatives (see material and methods, and Feuda et al. 2016).

We identified a total of 528 opsins across the 61 species (see Table S1 for the distribution of opsins in each species). We reconstructed their evolutionary affinities by Maximum Likelihood and Bayesian Inference (Figure 1, Figure S1, Figure S2) using the amino acid GTR-G model, which has previously been shown to fit opsin alignments better than other models (Feuda et al. 2012; Vöcking et al. 2017). Both approaches revealed that there are at least eight paralogous opsin groups in Diptera, which we named based on the *Drosophila* nomenclature as Rh1-7 and C. We also found that arthropsins and RGR/GO opsins have been lost in Diptera. Furthermore, both methods recover a similar topology,
whereby LWS opsins (Rh1, Rh2, and Rh6) are monophyletic (PP=1 and BS= 100) and Rh5 is the sister group to Rh3 plus Rh4 (PP=1 and BS=100). The position of Rh7 is uncertain, with Maximum Likelihood favouring their position as sister group to all the remaining R-opsins (BS=92, Figure S1). In summary, our phylogenetic analysis recovered the monophyly of all the main opsin groups (e.g., LWS, UV) with a high support value, which suggests the presence of eight opsin groups in dipterans.

**Dipteran opsins have undergone lineage-specific diversification**

To better understand the opsin distribution and evolutionary dynamics in the various dipteran groups, we mapped their presence/absence on the Diptera phylogeny (Figure 2A) and performed a manual as well as a statistical gene-tree/species-tree reconciliation (Figure 2B and 2C). The results indicate that the opsin repertoire underwent significant rearrangements on the dipteran phylogeny in a lineage-specific manner.

In Brachycera (the clade comprising *Drosophila*), the opsins complement is derived in comparison to the ancestral dipteran condition. We confirm previous findings that c-opsin and RGR/Go have been lost in all Brachycera (Feuda et al. 2016) and provide evidence that four paralogs - Rh1, Rh2, Rh3 and Rh4 - are present only in this group. The observation that at least one duplication from the ancestral Rh1/2/6 and Rh3/4 genes is shared between *Drosophila*, tephritid fruit flies, Muscidae house flies and Glossina tsetse flies indicates that these duplications happened early in Brachycera evolution (Figure 2B). We further observe various lineage-specific events, such as the loss of Rh4 in the common ancestor of Glossinidae, Muscidae and Calliphoridae, duplications of Rh1 in Muscidae, the loss of Rh2 in the tsetse fly *Glossina morsitans* (Attardo et al. 2019), and the loss of all opsins except for Rh2 and Rh6 in Diopside stalk-eyed flies. Interestingly, when we map introns’ presence/absence (Table S2) in the different opsins, the results indicate that Rh3 genes in all *Drosophila* species are intronless, suggesting their possible origin as retrotransposons (Booth & Holland 2004; Xu et al. 2016).

In the family Culicidae (mosquitoes, e.g., *Culex*, *Anopheles* and *Aedes*), opsins’ repertoire is markedly different from that observed in the Brachycera clade (Figure 2). For example, eight out of the 19 *Anopheles* species have a divergent copy of the Rh7 gene (Figure 1 and 2A), whose phylogenetic distribution suggests that it was present in the ancestral *Anopheles* and secondarily lost in some species. The most remarkable difference we observed between Brachycera and Culicidae is the impressive series of duplications of the Rh6 gene in the latter, where it ranges from three copies in *Anopheles melas* and *Anopheles christyi* to seven in *Culex quinquefasciatus*. We identified four Rh6 paralogs according to their relatedness (Figure 1) and distribution across the dipteran phylogeny (Figure 2B), which we named Rh6a, b, c and d. These duplications have already been identified in three Culicidae species (Giraldo-Calderón et al. 2017), but our data indicate that this pattern is present in all the sampled Culicidae species. This pattern of presence/absence suggests that at least two concomitant duplications of Rh6 happened in the Culicidae common ancestor, followed by additional lineage-specific duplications. To account for some *Anopheles* genomes characterised by low coverage genomes (Table S1), we further performed a manual search of all the missing genes to exclude the possibility of false negatives (see material and methods). Despite this careful manual curation, we could not untangle the precise evolutionary relationships of these duplications within *Anopheles*, because some species lack well-assembled and high-quality genomes (see Table S1). However, we found that, similar to Rh3 in *Drosophila*, multiple Rh6 paralogs lack introns (Table S2), suggesting that
these newly evolved genes may have originated from a retrotransposition event (Booth & Holland 2004; Xu et al. 2016).

Overall, our findings indicate that the opsin complement in the Brachycera and Culicidae is quite derived in comparison to ancestral dipteran, with the two groups having independently duplicated the LWS opsins. To further identify the ancestral opsin complements in key nodes (i.e. diptera, Brachyicerca, Drosophila, Culicidae and Anopheles), we performed a manual reconciliation as well as a gene tree–species tree reconciliation using the species tree obtained from the BUSCO single-copy orthologs and GeneRax (Morel et al. 2020) (see Methods for more details). The resulting ML tree recovers the traditional topology for diptera, except for the position of Psychodidae (Supplementary Figure S3). This tree, and a modified topology matching traditional dipteran relationships (Wiegmann et al. 2011), were used to reconcile the opsin phylogeny. In general, the computational reconciliations identified a similar pattern of duplications compared to the manual reconciliation (Figure 2C, Figure S4-S5). Most of the differences concern Rh6 in Anopheles, where GeneRax identified a large number of Rh6 copies. We think that this incongruence can be explained by the taxonomic levels used to perform the reconciliation (species vs genus) and the limited performance of GeneRax in dealing with incomplete lineage sorting (Morel et al. 2020), a phenomenon that is known to have shaped the mosquitos’ evolutionary history (Wen et al. 2016). We further tested the possible misleading effect of incomplete genomes by repeating the gene-tree species tree reconciliation after removal of all the species with a BUSCO Value < 85%. The results (Figure S6) suggest that for the key nodes of Figure 2C, there are no differences between the two datasets.

The evolution of opsin genes in Drosophila, Aedes and Anopheles species

Our reconciliation analyses indicate that starting from a repertoire of five (or nine according to GeneRax) opsin genes, the complement substantially diverged in the Brachycera clade compared to the Culicidae family, with several lineage-specific events (Figure 2B). The question arises as to whether these newly duplicated genes are expressed in photoreceptor cells and are associated with divergence and specialisation of the visual system. In D. melanogaster, there is ample evidence that all opsin genes, including the newly duplicated intronless Rh3, are expressed and functional in photoreceptor cells and combinatorially define the different visual neural circuits (Courgeon & Desplan 2019).

However, the expression of opsin genes in other cells of the visual system remains poorly understood. We then investigated the pattern of opsin expression in other cell types of the Drosophila’s optic lobe by mining single-cell RNA-seq data previously obtained from Davis and collaborators (Davis et al. 2020). Our data indicate that opsins expression is not restricted to the photoreceptor cells and that they contribute to different aspects of the visual neural circuits. For example, the Rh7 mRNA is detected in the lamina neurons L1-2 (Figure S7A) that regulate motion. Furthermore, the function of the newly duplicated opsin genes in D. melanogaster may not be restricted to the visual process: for instance, it has been recently been proposed that Rh1, Rh4 and Rh7 are involved in taste (Leung & Montell 2017; Leung et al. 2020), suggesting a co-option of visual genes in different sensory pathways. In mosquitos, the information on opsin gene expression is scant. However, it is interesting to note that the R7 photoreceptor of Aedes aegypti may express, depending on their actual position in the retina, the LWS (Rh6a-AAop2 or Rh7-Aaop10), the SWS Aaop9 (Rh5) and the UV-(Rh3-Aaop8) opsins (Rocha et al. 2015; Hu et al. 2014). We further investigated the expression of opsin genes in Anopheles gambiace and Aedes aegypti by analysing available microarray datasets (Baker et al. 2011; Leming et al. 2014).
The results indicate that \textit{Rh6a}, \textit{Rh3/4} and \textit{Rh5} are statistically over-expressed in the head of \textit{Anopheles gambiae} (Table S3), while all nine opsins we identified in \textit{Aedes aegypti} are expressed in the head (See Figure S7B). While this expression data is not eye-specific, these results suggest that these opsins are potentially expressed and contribute to the mosquitos' visual system. We advocate that more specific gene expression studies (focused on the eye rather than on the whole head) are necessary to determine with confidence whether these genes are actually being expressed in the eye and if they have a role in colour vision.

\textbf{Opsins in \textit{Drosophila} and \textit{Anopheles} underwent substantial divergent molecular evolution}

Our results indicate that the opsin complement is quite divergent across the various dipteran families. We then asked if the pattern of opsin evolution also differs within different genera. To maximise the power of our analyses and inferences, we focused on two genera for which we had around 20 genomes each: \textit{Drosophila} and \textit{Anopheles}. Interestingly, while all \textit{Drosophila} species have exactly the same opsin complement, indicating a frozen repertoire over circa 60 million years, the similarly aged \textit{Anopheles} genus is characterised by an extremely plastic opsin repertoire that includes lineage-specific duplications of \textit{Rh7} (\textit{Rh7}-like) and \textit{C} opsin, and various instances of duplications and losses of \textit{Rh6} (Figure 2 and Table S1).

To clarify the pattern of selection acting on the opsin genes in these two dipteran genera, we produced manually curated opsin alignments for each paralog group and estimated the selective pressure using PAML (see Material and Methods for details). Importantly, the cross-group comparison is possible because both these two genera have a similar evolutionary history: both emerged in the Paleogene (between 100 and 30 mya according to Neafsey et al. 2015; Obbard et al. 2012, Ometto et al. 2013) and have a similar number of generations per year (up to 10). We found that the differences between \textit{Drosophila} and \textit{Anopheles} are not restricted to the opsin repertoire alone but extend to the pattern of molecular evolution of the opsin genes. While these two groups show an unusual signature of selection for a similar number of genes (26 and 23 respectively, in colour in Figure 3A and 3B), in \textit{Anopheles}, we observe more events of site-specific positive selection (1 in \textit{Drosophila} and 7 in \textit{Anopheles}, magenta squares in Figure 3A and 3B). A second difference concerns the rate of amino acid evolution. Opsi n genes are subject to different molecular constraints in the two groups, as supported by a slightly lower selective pressure in \textit{Anopheles} than in \textit{Drosophila} (Figure 3E and 3F; overall $d_{s}/d_{a} = 0.0573$ and $d_{s}/d_{a} = 0.0374$, respectively). This is because while the two clades are characterised by a similar rate of synonymous nucleotide substitution (on average $d_{s} = 0.2012$ and $d_{s} = 0.1969$, respectively; data not shown), the two are characterised by different non-synonymous rates (Figure 3C and 3D; on average $d_{a} = 0.0118$ in \textit{Anopheles} and $d_{a} = 0.0073$ in \textit{Drosophila}).

Our molecular evolution results indicate a much higher variability in selective pressure across opsin genes in \textit{Anopheles} than in \textit{Drosophila}. These different evolutionary patterns are independent of data treatment: when regions with gaps are removed from the alignments (Figures S8, S9, S10), we observe lower substitution rates in \textit{Anopheles} (because the orthologs in this genus are less constrained and accumulated more indels), but trends are consistent. Overall, our results indicate that in the genus \textit{Anopheles} the opsin genes experienced a different evolutionary path and were subject to an accelerated rate of evolution compared to the \textit{Drosophila} species. This is consistent and complementary with the more dynamic pattern of gene deletions/duplications we identified in
Anopheles. While almost all Drosophila species are diurnal, Anopheles can be both nocturnal and/or crepuscular (Table S1), suggesting that their extremely flexible opsin repertoire is playing an active role in their adaptation to different lifestyles. Importantly, our results do not allow us to determine whether the differences in the selective pressures are indicative of actual selective forces happening in the visual system (e.g., spectral tuning) or in the other sensory modalities.

Conclusion and future perspectives

Here we have characterized the evolutionary history of the opsin genes in ten dipteran families, focusing on the fine-scaled molecular evolution of model organisms Drosophila and Anopheles. Overall, we found that different dipterans underwent distinct patterns of deletions/duplications (Figure 1 and 2) and positive selection (Figure 3). One of the key findings is the derived complement (Rh1, Rh2, Rh3 and Rh4) of the Brachycera species, including the model organism Drosophila. These genes' recent evolutionary origin suggests that the non-visual opsin function in Drosophila (Leung & Montell 2017; Leung et al. 2020) probably represents a lineage-specific co-option event (Pisani et al. 2020) and implies that Drosophila's opsins cannot be used to infer the ancestral function of these genes (Leung et al. 2020). Our data indicates that the opsin complement is even more dynamic in mosquitos, particularly concerning Rh6 and Rh7. Moreover, our analyses revealed that the Anopheles lineages had experienced more instances of site-specific selective pressure and faster evolutionary rates than the Drosophila lineages (Figure 3).

In the absence of functional studies, it is impossible to assign an unequivocal role to the pattern of duplication and positive selection we have identified. However, our results allow us to formulate working hypotheses that can be experimentally tested in future studies. For example, the high heterogeneity in the selective pressure acting on Drosophila Rh7 (Figure 3E), coupled with its fast evolution (i.e., the high $d_{ns}$, Figure 3C) and expression in the clock-neurons (Ma et al. 2021) may be associated with divergence in the circadian clock in species with different ecology and latitudinal distribution (Menegazzi et al. 2017). This might explain the findings in the agricultural pest Drosophila suzukii, a species characterised by significant selective pressure affecting Rh6 and Rh7 (Figure 3). These changes are interesting from an applicative point of view, as it is possible that they are linked to the peculiar circadian activity (Hansen et al. 2019), colour recognition pattern (Little et al. 2019), and even gustatory preferences (Crava et al. 2016; Leung et al. 2020) associated with this species' peculiar ecological lifestyle. In Anopheles, opsin function is less well understood than Drosophila (Montell & Zwiebel 2016). However, different mosquito species may be active during specific periods of the day or night, when light is characterised by a different wavelength composition (Downes 1969; Sawadogo et al. 2014). The opsins' unique capacity to tune their maximum absorbance to specific light conditions might therefore have had a role in these ecological differences (Jenkins & Muskavitch 2015). Indeed, we hypothesise that the high variability in the selective pressure affecting Rh7 and C-opsin in the Anopheles species (Figure 3F) may be linked to differences in their adaptation to light detection, including the possible function in the circadian clock.

Future works should concentrate on the physiological significance of the duplication/losses we have identified, as well as seeking to understand the functional role of the sites under positive selection. This requires, for example: 1) the validation of the candidate's selected sites using PCR; 2) a 3D reconstruction of the various opsins, which is complicated by the high divergence between orthologs.
and the absence of a validated 3D structure for most of the opsins; 3) site-specific mutants to validate any possible function. Overall, our work serves as a comparative genomic overview of opsin evolution in dipterans and represents the foundation for future studies aimed at improving our understanding of dipteran visual biology and the management of economically relevant species such as mosquitoes (e.g., Zhan et al. 2020) and fruit-eating flies.

MATERIALS AND METHODS

Opsin identification. We downloaded 61 predicted proteomes from 10 Diptera families (Culicidiae, Chironimidiae, Psychodidiae, Drosophilidae, Tephritidae, Glossinidae, Calliphoridae, Muscidae, Diopsidae, Ceciomyiidae, Table S1). We evaluated their quality by assessing their completeness with BUSCO (Simão et al. 2015), using the 1,367 single-copies orthologs of the insects lineage dataset. To identify the opsin genes, we employed a combination of BLAST and motif search similarly to Feuda et al. (2016). In brief, the sequences from Feuda et al. (2016, 2012) and Ramirez et al. (2016) were used to mine every genome. From this analysis, every gene with an e-value < 10^{-10} was retained as a putative opsin gene and was subject to a motive search using Prosite (Sigrist et al. 2013) and an annotation using BLASTP against the Uniprot90 Database. To be considered an opsin, either one of two conditions was sufficient: the sequence must contain a retinal binding domain or have an opsin as the first hit in the BLAST search. Using this approach and after a preliminary manual annotation, we identified 528 opsin genes (Table S1). Alignment and trees are available on Bitbucket (https://bitbucket.org/Feuda-lab/opsin_diptera/src/master/).

Manual curation. The dataset obtained was eventually manually curated. For example, we first checked for missing data. We selected sequences that lacked part of the opsin protein, and, where possible, we retrieved the missing data using BLAST (tblastn) on the assembled genomes. Second, we looked for putative false duplications in the tree, and in the case where we found a species-specific duplication in our subsequent analyses, we removed the incomplete sequence. Moreover, we looked for unexpected opsin losses to assess whether the missing genes were true losses or artefacts (false negatives). In some cases, we found the missing gene in the genome of interest and the sequence was added manually to the alignment.

For some mosquito species, we lacked well-assembled genomes and, therefore, accurate gene models, which may have caused misrepresentation in the exact number of Rh6 copies in each Anopheles lineage and blurred the fine-scale duplications/losses pattern. We therefore carefully and manually validated the Rh6 genes in the Anopheles species. Using such an approach allowed us to increase the length of many orthologs, most importantly, allowing us to detect instances of false positives: cases where putative duplicated contigs or allele variants from heterozygotes genomes could be mistaken for species-specific duplications.

We further manually inspected for possible pseudogenes. For the Drosophila and Anopheles species we manually curated all the alignments in order to perform dW/dS studies (see below) to exclude pseudogenes, because we could not find signature of pseudogenes (dW/dS=1), nor we detect internal stop codons. For all other species we inspected the alignment by eye when the gene was characterized by extremely long branches.
Gene structure characterization. We investigated intron presence in the 61 dipteran species under study using Vector base (Giraldo-Calderón et al. 2015), Ensembl (Yates et al. 2020), and in some cases by manual curation. The full gene region of each of the seven opsins in Drosophila was further inspected in the FlyBase genome browser for detailed intron length, which was mapped separately in Table S3. To assess significant events of intron length variation, we developed a method which assumed a normal distribution for the length of each intron and highlighted significant introns whose length was larger (or shorter) than the mean plus twice the standard deviation for that intron (estimated excluding from the target intron).

Phylogenetic analysis. To identify the phylogenetic relationships between the opsin genes, we performed a phylogenetic analysis using Bayesian and Maximum Likelihood inferences. The opsins dataset was first merged to a subsample of the insect dataset of Feuda et al. (2016), and the sequences aligned using MAFFT v7.4 (Katoh et al. 2002) with default parameters. The maximum likelihood tree was performed using IQTree 2.0 (Nguyen et al. 2015) under the GTR-G4 model. The Bayesian tree was performed using Phylobayes-MPI (Lartillot et al. 2013) under the GTR-G4 model and node support was estimated using Posterior Probability (PP).

GeneRax analysis. We used the 1367 BUSCO single-copy orthologs (see above) to assemble a supermatrix composed of 505,000 amino acid positions. The species tree was estimated using the single-copy gene hits from the BUSCO analyses (see above). The sequences of each BUSCO gene were extracted and aligned using MAFFT v7.4 (Katoh et al. 2002) and trimmed using gblocks v0.91 (Talavera & Castresana 2007) (allowing half gaps, minimum block length 5, maximum contiguous nonconserved positions 5 and 75% of sequences present in flank positions), then all alignments were concatenated using FASconcat v1.11 (Kuck & Meusemann 2010). The concatenated alignment consisted of 73 species (61 Diptera, 4 Lepidoptera) with 504,666 nucleotide positions and was analyzed under LG+F+I+G4. Both selection and phylogenetic inference were performed in IQ-TREE2 (Nguyen et al. 2015). This species tree (FigureS3), and a manually modified version matching that presented in Figure 2 and Wiegmann et al. (2011), were used for species tree-gene tree reconciliation analysis using GeneRax (Morel et al. 2020) alongside the opsin gene tree resolved using GTR-G (Figure S2). Reconciled trees are displayed in Figure S4, S5 and S6. Alignment and trees are available on bitbucket (https://bitbucket.org/Feuda-lab/opsin_diptera/src/master/).

Positive selection. The coding sequence of each opsin subgroup (Rh1, Rh2, Rh3, Rh4, Rh5, Rh6, Rh7, and C) were aligned separately for the 21 Drosophila and 19 Anopheles species using the PRANK (Loytynoja & Goldman 2008) codon model, which produces fewer false-positives in positive selection analysis (Markova-Raina & Petrov 2011). Each alignment was manually curated to avoid spurious divergence signals that may have biased the subsequent analyses, and we generated two sets of alignments, one using all sites and a second where all the regions containing gaps were removed. We inferred the level of selective pressure acting on each of the 7 Drosophila opsins using PAML 4.7 (Yang 2007). Rates of synonymous (dS) and nonsynonymous substitution (dN), as well as their ratio ω = dN/dS (which measures levels of selective pressure acting on a gene), were estimated by the “free-ratio” model using the unrooted species tree topology inferred above. In this analysis, alignments included only sequences from those species that were represented in all opsin alignments to allow cross opsin-gene comparisons in Anopheles. Heterogeneity in the selective pressure was inferred using a branch-test to compare the likelihood of a single ω model across branches (model = 0 and Ns sites = 0) versus one assuming two distinct ω, one for each terminal branch, one at a time (i.e., for each Drosophila
and Anopheles species in their respective datasets), and another for rest of the tree. To further identify the occurrence of positive selection on specific sites we employed the branch-site test (branch-site model A, test 2; model = 2 and NS sites = 2; null model has parameters fix_\(\omega\) = 1, \(\omega\) = 1; the positive selection model fix_\(\omega\) = 0, \(\omega\) = 1, with each species set as foreground species in separate analysis, see above). Both tests were estimated using either the whole alignment (clean = 0) or removing parts of the alignment where one or more sequences contained a gap (clean=1). We tested twice the difference between the log-likelihood of the two models for both tests using a \(\chi^2\) test with 1 degree of freedom. To account for multiple testing, we estimated the false discovery rate (FDR) of each test using the q-value approach (Storey 2002) implemented in R (R Development Core Team, 2009). All statistics are summarised in Table S4.
**Figure 1. The phylogenomics of opsins in Diptera.** This figure shows the phylogenetic relationships between 528 opsins from 61 dipteran species. The Bayesian Posterior Probability (in bold) and the UltraFast bootstrap (underlined) support is shown for key nodes. The colours indicate the different Diptera subgroups analyzed in this work. Letters A, B, C and D indicate the different paralogs of $Rh6$ we identified in Culicidae. The full trees can be found in Figure S1 and S2.

**Figure 2. Opsins evolution in Diptera.** (A) Opsin gene complements in Diptera. The phylogenetic tree was obtained from Wiegmann et al. (2011). Gene nomenclature has been obtained from *Drosophila melanogaster*. The numbers in the boxes indicate the copies of opsin genes identified; white boxes indicate that genes have not been found. (B) Synopsis of the patterns of opsin duplications and losses in Diptera subgroups. Lineage specific events are marked with a question mark if they were inferred from one single representative genome. (C) Estimated number of ancestral Rh across five nodes. For each opsin paralog, we report the estimate using three different analytical procedures (manual reconciliation, GeneRax on tree of Figure 2A, GeneRax on tree of Figure S3).

**Figure 3. Pattern of positive selection and molecular evolution of the opsin genes in *Drosophila* and *Anopheles*.** Genes under selection according to the branch or branch-site tests in one species of *Drosophila* and *Anopheles* are shown in panels (A) and (B), respectively. We also report the rate of protein evolution ($d_{ni}$) and the level of selective pressure ($d_{ni}/d_{is}$) across opsin phylogenies in *Drosophila* (panels C and E) and *Anopheles* (panels D and F). Genes are strong determinants of the variance in $d_{ni}$ and $d_{ni}/d_{is}$ values in both *Drosophila* (ANOVA, $F(6,266) = 7.43$, $P < 10^{-6}$; and $F(6,259) = 4.88$, $P = 0.0001$, respectively) and *Anopheles* (ANOVA, $F(8,126) = 6.37$, $P < 10^{-6}$; and $F(8,124) = 8.60$, $P < 10^{-8}$, respectively). Different letters identify significant statistical differences between genes at adjusted $P < 0.05$, according to a Tukey's HSD (honestly significant difference) multiple comparison test. Median and quantiles are shown as grey lines for each gene. These analyses were performed including parts of the alignments where one or more sequences contained a gap. FDR = False Discovery Rate. Detailed information for each gene is in Table S4.

**Data availability**

The data underlying this article are available on bitbucket (https://bitbucket.org/Feuda-lab/opsin_diptera/src/master/).

**Author contributions**

R.F. and O.R.S. conceived the study. R.F., M.G., N.Z., L.O., and O.M.S. performed computational analyses. R.F., M.G., T.G., E.R., N.S., A.R., D.P., L.O., and O.R.S. performed the data interpretation. R.F. and O.R.S. wrote the main text with the help of L.O., and inputs from all authors.

**ACKNOWLEDGMENTS**

This study was supported by a Royal Society University Research Fellowship (UF160226) to R.F.

**REFERENCES**

Almudi I et al. 2020. Genomic adaptations to aquatic and aerial life in mayflies and the origin of insect wings. Nature Communications. 11:2631. doi: 10.1038/s41467-020-16284-8.
Anstead CA et al. 2016. A blow to the fly - Lucilia cuprina draft genome and transcriptome to support advances in biology and biotechnology. Biotechnol Adv. 34:605–620. doi: 10.1016/j.biotechadv.2016.02.009.

Attardo GM et al. 2019. Comparative genomic analysis of six Glossina genomes, vectors of African trypanosomes. Genome Biol. 20:187. doi: 10.1186/s13059-019-1768-2.

Attardo GM et al. 2014. Genome sequence of the tsetse fly (Glossina morsitans): vector of African trypanosomiasis. Science. 344:380–386.

Baker DA et al. 2011. A comprehensive gene expression atlas of sex- and tissue-specificity in the malaria vector, Anopheles gambiae. BMC Genomics. 12:296. doi: 10.1186/1471-2164-12-296.

Bao R, Friedrich M. 2009. Molecular Evolution of the Drosophila Retinome: Exceptional Gene Gain in the Higher Diptera. Molecular Biology and Evolution. 26:1273–1287. doi: 10.1093/molbev/msp039.

Booth HAF, Holland PW. 2004. Eleven daughters of NANOG. Genomics. 84:229–238.

Briscoe AD, Chittka L. 2001. The evolution of color vision in insects. Annual review of entomology. 46:471–510.

Carulli JP, Chen D-M, Stark WS, Hartl DL. 1994. Phylogeny and physiology of Drosophila opsins. Journal of molecular evolution. 38:250–262.

Courgeon M, Desplan C. 2019. Coordination of neural patterning in the Drosophila visual system. Current Opinion in Neurobiology. 56:153–159. doi: 10.1016/j.conb.2019.01.024.

Crava CM, Ramasamy S, Ometto L, Anfora G, Rota-Stabelli O. 2016. Evolutionary Insights into Taste Perception of the Invasive Pest Drosophila suzukii. G3 (Bethesda). 6:4185–4196. doi: 10.1534/g3.116.036467.

Davis FP et al. 2020. A genetic, genomic, and computational resource for exploring neural circuit function Bellen, HJ & VijayRaghavan, K, editors. eLife. 9:e50901. doi: 10.7554/eLife.50901.

Downes JA. 1969. The Swarming and Mating Flight of Diptera. Annu. Rev. Entomol. 14:271–298. doi: 10.1146/annurev.en.14.010169.001415.

Duchemin W et al. 2018. RecPhyloXML: a format for reconciled gene trees. Bioinformatics. 34:3646–3652. doi: 10.1093/bioinformatics/bty389.

Fain GL, Hardie R, Laughlin SB. 2010. Phototransduction and the evolution of photoreceptors. Current Biology. 20:R114-24. doi: S0960-9822(09)02125-3 [pii] 10.1016/j.cub.2009.12.006.

Feuda R, Marletaz F, Bentley MA, Holland PW. 2016. Conservation, duplication, and divergence of five opsin genes in insect evolution. Genome biology and evolution. 8:579–587.

Fleming JF et al. 2018. Molecular palaeontology illuminates the evolution of ecdysozoan vision. Proceedings of the Royal Society B: Biological Sciences. 285:20182180. doi: 10.1098/rspb.2018.2180.

Futahashi R et al. 2015. Extraordinary diversity of visual opsin genes in dragonflies. Proceedings of the National Academy of Sciences. 112:E1247–E1256.

Giraldo-Calderón GI et al. 2015. VectorBase: an updated bioinformatics resource for invertebrate vectors and other organisms related with human diseases. Nucleic Acids Res. 43:D707-713. doi: 10.1093/nar/gku1117.

Giraldo-Calderón GI, Zanis MJ, Hill CA. 2017. Retention of duplicated long-wavelength opsins in mosquito lineages by positive selection and differential expression. BMC Evolutionary Biology. 17:84. doi: 10.1186/s12862-017-0910-6.

Hansen CN et al. 2019. Locomotor Behaviour and Clock Neurons Organisation in the Agricultural Pest Drosophila suzukii. Front. Physiol. 10. doi: 10.3389/fphys.2019.00941.

Henze MJ, Oakley TH. 2015. The dynamic evolutionary history of pancrustacean eyes and opsins. Integrative and comparative biology. 55:830–842.

Hu X, Leming MT, Whaley MA, O’Tousa JE. 2014. Rhodopsin coexpression in UV photoreceptors of Aedes aegypti and Anopheles gambiae mosquitoes. Journal of Experimental Biology. 217:1003–1008. doi: 10.1242/jeb.096347.
Jenkins AM, Muskavitch MAT. 2015. Crepuscular Behavioral Variation and Profiling of Opsin Genes in Anopheles gambiae and Anopheles stephensi (Diptera: Culicidae). J Med Entomol. 52:296–307. doi: 10.1093/jme/jtv024.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30:3059–66.

doi: 10.1093/jme/tjv024.

van der Kooi CJ, Stavenga DG, Arikawa K, Belušič G, Kelber A. 2021. Evolution of Insect Color Vision: From Spectral Sensitivity to Visual Ecology. Annu. Rev. Entomol. 66:435–461. doi: 10.1146/annurev-ento-061720-071644.

Kuck P, Meusemann K. 2010. FASconCAT: Convenient handling of data matrices. Mol Phylogenet Evol. 56:1115–8. doi: 10.1016/j.ympev.2010.04.024.

Lartillot N, Rodrigue N, Stubbs D, Richer J. 2013. PhyloBayes MPI: phylogenetic reconstruction with infinite mixtures of profiles in a parallel environment. Syst Biol. 62:611–5. doi: 10.1093/sysbio/syt022.

van der Kooi CJ, Stavenga DG, Arikawa K, Belušič G, Kelber A. 2021. Evolution of Insect Color Vision: From Spectral Sensitivity to Visual Ecology. Annu. Rev. Entomol. 66:435–461. doi: 10.1146/annurev-ento-061720-071644.

Kuck P, Meusemann K. 2010. FASconCAT: Convenient handling of data matrices. Mol Phylogenet Evol. 56:1115–8. doi: 10.1016/j.ympev.2010.04.024.

Lartillot N, Rodrigue N, Stubbs D, Richer J. 2013. PhyloBayes MPI: phylogenetic reconstruction with infinite mixtures of profiles in a parallel environment. Syst Biol. 62:611–5. doi: 10.1093/sysbio/syt022.

Leung NY et al. 2020. Functions of Opsins in Drosophila Taste. Current Biology. doi: 10.1016/j.cub.2020.01.068.

Leung NY, Montell C. 2017. Unconventional roles of opsins. Annu Rev Cell Dev Biol. 33:241–264. doi: 10.1146/annurev-cellbio-100616-060432.

Little CM, Rizzato AR, Charbonneau L, Chapman T, Hillier NK. 2019. Color preference of the spotted wing Drosophila, Drosophila suzukii. Scientific Reports. 9:1–12. doi: 10.1038/s41598-019-52425-w.

Lemytynoja A, Goldman N. 2008. Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis. Science. 320:1632–5. doi: 10.1126/science.1158395.

Lemming MT, Rund SS, Behura SK, Duffield GE, O’Toosa JE. 2014. A database of circadian and diel rhythmic gene expression in the yellow fever mosquito Aedes aegypti. BMC Genomics. 15:1128. doi: 10.1186/1471-2164-15-1128.

Loytynoja A, Goldman N. 2008. Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis. Science. 320:1632–5. doi: 320/5883/1632 [pii] 10.1126/science.1158395.

Ma D et al. 2021. A transcriptomic taxonomy of Drosophila circadian neurons around the clock. Elife. 10. doi: 10.7554/eLife.63056.

Markova-Raina P, Petrov D. 2011. High sensitivity to aligner and high rate of false positives in the estimates of positive selection in the 12 Drosophila genomes. Genome research. 21:863–874.

Menegazzi P et al. 2017. Adaptation of Circadian Neuronal Network to Photoperiod in High-Latitude European Drosophilids. Curr. Biol. 27:833–839. doi: 10.1016/j.cub.2017.01.036.

Montell C, Zwiebel LJ. 2016. Chapter Ten - Mosquito Sensory Systems. In: Advances in Insect Physiology. Raikhel, AS, editor. Progress in Mosquito Research Vol. 51 Academic Press pp. 293–328. doi: 10.1016/bs.aiip.2016.04.007.

Morel B, Kozlov AM, Stamatakis A, Zöllösi GJ. 2020. GeneRax: A Tool for Species-Tree-Aware Maximum Likelihood-Based Gene Family Tree Inference under Gene Duplication, Transfer, and Loss. Molecular Biology and Evolution. 37:2763–2774. doi: 10.1093/molbev/msaa141.

Neafsey DE et al. 2015. Mosquito genomics. Highly evolvable malaria vectors: the genomes of 16 Anopheles mosquitoes. Science. 347:1258522. doi: 10.1126/science.1258522.

Ni JD, Baik LS, Holmes TC, Montell C. 2017. A rhodopsin in the brain functions in circadian photoentrainment in Drosophila. Nature. 545:340–344. doi: 10.1038/nature22325.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 32:268–74. doi: 10.1093/molbev/msu300.

Pollock JA, Benzer S. 1988. Transcript localization of four opsin genes in the three visual organs of Drosophila; RH2 is ocellus specific. Nature. 333:779–782. doi: 10.1038/333779a0.
Ramirez MD et al. 2016. The Last Common Ancestor of Most Bilateral Animals Possessed at Least Nine Opsins. Genome Biol Evol. 8:3640–3652. doi: 10.1093/gbe/eww248.

Rocha M et al. 2015. Expression and light-triggered movement of rhodopsins in the larval visual system of mosquitoes. Journal of Experimental Biology. 218:1386–1392. doi: 10.1242/jeb.111526.

Rota-Stabelli O et al. 2020. Distinct genotypes and phenotypes in European and American strains of Drosophila suzukii: implications for biology and management of an invasive organism. J Pest Sci. 93:77–89. doi: 10.1007/s10340-019-01172-y.

Sakai K et al. 2017. Drosophila melanogaster rhodopsin Rh7 is a UV-to-visible light sensor with an extraordinarily broad absorption spectrum. Scientific Reports. 7:1–11. doi: 10.1038/s41598-017-07461-9.

Sawadogo PS et al. 2014. Swarming behaviour in natural populations of Anopheles gambiae and An. coluzzii: review of 4 years survey in rural areas of sympathy, Burkina Faso (West Africa). Acta Trop. 132 Suppl:S42–52. doi: 10.1016/j.actatropica.2013.12.011.

Schmitt A, Vogt A, Friedmann K, Paulsen R, Huber A. 2005. Rhodopsin patterning in central photoreceptor cells of the blowfly Calliphora vicina: cloning and characterization of Calliphora rhodopsins Rh3, Rh5 and Rh6. Journal of Experimental Biology. 208:1247–1256. doi: 10.1242/jeb.01527.

Sigrist CJA et al. 2013. New and continuing developments at PROSITE. Nucleic Acids Res. 41:D344-347. doi: 10.1093/nar/gks1067.

Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 31:3210–3212. doi: 10.1093/bioinformatics/btv351.

Skevington JH, Dang PT. 2002. Exploring the diversity of flies (Diptera). Biodiversity. 3:3–27. doi: 10.1080/14888386.2002.9712613.

Sokabe T, Chen H-C, Luo J, Montell C. 2016. A Switch in Thermal Preference in Drosophila Larvae Depends on Multiple Rhodopsins. Cell Reports. 17:336–344. doi: 10.1016/j.celrep.2016.09.028.

Sondhi Y, Ellis EA, Theobald JC, Kawahara AY. 2020. Light environment drives evolution of color vision genes in butterflies and moths. bioRxiv. 2020.02.29.965335. doi: 10.1101/2020.02.29.965335.

Storey JD. 2002. A direct approach to false discovery rates. Journal of the Royal Statistical Society: Series B (Statistical Methodology). 64:479–498. doi: 10.1111/1467-9868.00346.

Talavera G, Castresana J. 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol. 56:564–77. doi: 10.1080/10635150701472164.

Tierney SM et al. 2015. Opsin transcripts of predatory diving beetles: a comparison of surface and subterranean photic niches. Royal Society Open Science. 2:140386.

Tierney SM et al. 2011. Photic niche invasions: phylogenetic history of the dim-light foraging augochlorine bees (Halictidae). Proceedings of the Royal Society of London B: Biological Sciences. rspb20111355.

Velarde RA, Sauer CD, Walden KK, Fahrbach SE, Robertson HM. 2005. Pteropsin: a vertebrate-like non-visual opsin expressed in the honey bee brain. Insect biochemistry and molecular biology. 35:1367–1377.

Vöcking O, Kourtesis I, Tumu SC, Hausen H. 2017. Co-expression of xenopsin and rhabdomeric opsin in photoreceptors bearing microvilli and cilia. Elife. 6. doi: 10.7554/elife.23435.

Wen D, Yu Y, Hahn MW, Nakhleh L. 2016. Reticulate evolutionary history and extensive introgression in mosquito species revealed by phylogenetic network analysis. Mol Ecol. 25:2361–2372. doi: 10.1111/mec.13544.

White IM, Elson-Harris MM. 1992. Fruit flies of economic significance: their identification and bionomics. Fruit flies of economic significance: their identification and bionomics. https://www.cabdirect.org/cabdirect/abstract/19921161954 (Accessed April 8, 2020).

Wiegmann BM et al. 2011. Episodic radiations in the fly tree of life. PNAS. 108:5690–5695. doi: 10.1073/pnas.1012675108.
Xu P et al. 2016. Functional opsin retrogene in nocturnal moth. Mobile DNA. 7:18. doi: 10.1186/s13100-016-0074-8.

Yang Z. 2007. PAML 4: phylogenetic analysis by maximum likelihood. Molecular biology and evolution. 24:1586–1591.

Yates AD et al. 2020. Ensembl 2020. Nucleic Acids Res. 48:D682–D688. doi: 10.1093/nar/gkz966.

Zadra N, Rizzoli A, Rota-Stabelli O. 2021. Chronological Incongruences between Mitochondrial and Nuclear Phylogenies of Aedes Mosquitoes. Life. 11:181. doi: 10.3390/life11030181.

Zanini D et al. 2018. Proprioceptive Opsin Functions in Drosophila Larval Locomotion. Neuron. 98:67-74.e4. doi: 10.1016/j.neuron.2018.02.028.

Zhan Y, Alberto DAS, Rusch C, Riffell JA, Montell C. 2020. Aedes aegypti vision-guided target recognition requires two redundant rhodopsins. bioRxiv. 2020.07.01.182899. doi: 10.1101/2020.07.01.182899.
