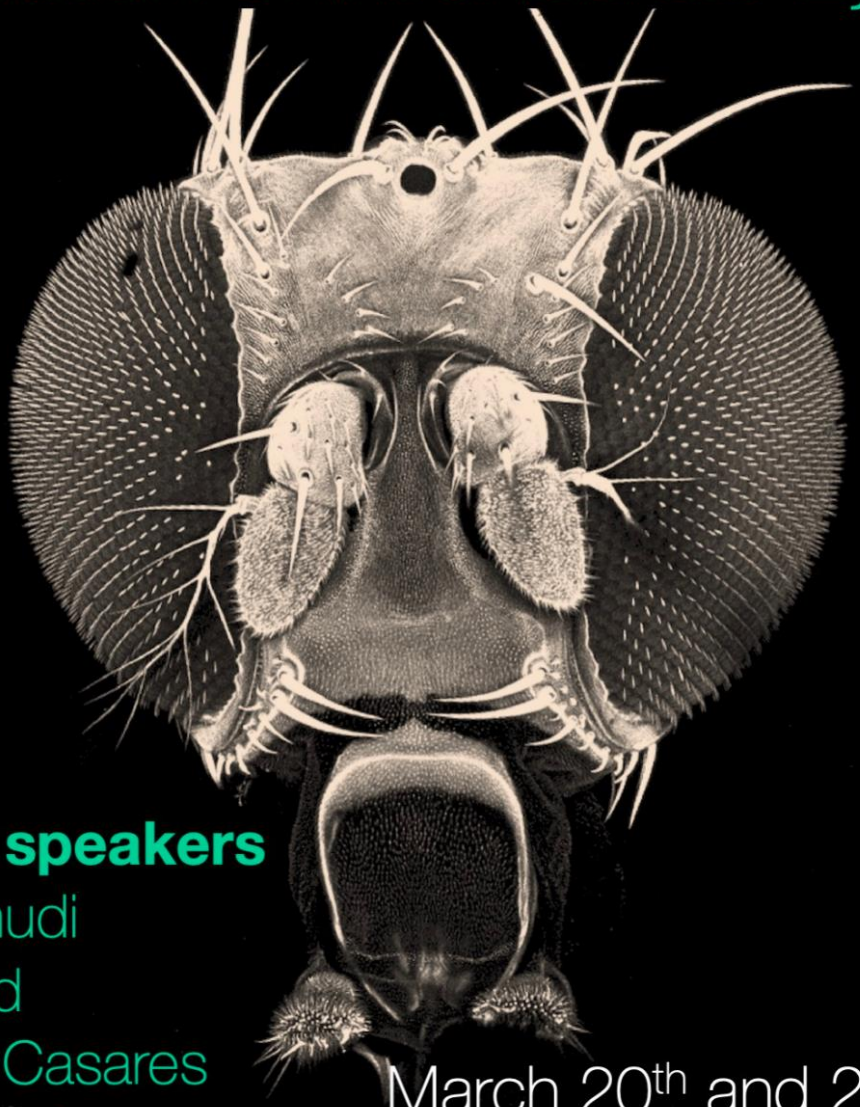
Development, Function, and Evolution of Invertebrate Eyes



Keynote speakers

Isabel Almudi

Emily Baird

Fernando Casares

Workshop by

Pablo Currea

March 20th and 21st 2023

Göttingen, Germany

Register by 20th Feb 23

Organisers

Alex Buffry

Nico Posnien

Maike Kittelmann

Alistair McGregor

Lauren Sumner-Rooney

https://www.posnien-lab.net/eye-meeting/

Supported by:

Schedule

Monday 20th March		
1.30 pm		Registration and welcome
	Session 1: Evolution	
2pm	**Isabel Almudi**	**Development, function and evolution of turbanate eyes of mayflies**
2.30pm	Gregor Belušič	Evolution of red receptors in nymphalid butterflies
2.45pm	Peter Mulhair	Many modes of opsin evolution in *Lepidoptera*
3.00pm	Atal Pande	Visual Allometry in Earwigs (*Dermaptera*)
3.04pm	Alison Irwin	Evolution of large eyes in the conch snail group *Stromboidea*
3.08pm		*Coffee*
3.40pm	Stefan Graf	Comparative morphology of modified ocelli in apoid wasps
3.44pm	Andre Ampuero-Leon	To see beyond the shell: Sensory organs in chitons (Mollusca: Polyplacophora)
3.48pm	Lauren Sumner-Rooney	The evolution of eye size in spiders
4.03pm		*Break*
4.15pm	**Pablo Currea**	**Workshop: Predicting vision from morphology**
6pm		*End and transfer to city center*
7pm		*Dinner: Bullerjahn, Goettingen*

Tuesday 21st March

Session 2: Function

9.30am	**Emily Baird**	**What does a bee see? Imaging the visual world of bees to explore the link between vision and flight**
10am	Heidi Roth	Skylight navigation across insects- evolutionary mechanisms optimizing neural circuit structure and function
10.15am	Jérôme Delroisse	From molecules to behaviours: insights into opsin-based light perception in echinoderms
10.30am	Lena Stanislawczyk	ts-CRISPR-induced knockdown of Rab proteins leads to defects in TRPL transport in *Drosophila* photoreceptor cells
10.34am	Pavel Kviatko	Insect retinal muscles in the context of visual ecology
10.38am		*Coffee*
11.10am	Alejandro Martín	Spectral sensitivity of tortricid moths is not tuned to diel activity period
11.25am	Pedro Domingos	Xport-A functions as a chaperone by stabilizing the first 5 transmembrane domains of Rhodopsin-1
11.40pm	Youri Nonclercq	New lights on ocellar and extraocellar photoreception in sea cucumbers (*Holothuroidea, Echinodermata*)
11.55pm	Pingkalai Senthilan	Rhodopsin 7 in *Drosophila melanogaster*
12.10pm		*Lunch*

Session 3: Development

2pm	**Fernando Casares**	**Variations on a theme**
2.30pm	Lucas Di Pietro N Figueiredo Pinto	Using transcriptomics to identify regulators of the subdivision of early *Drosophila* eye-antennal imaginal discs: challenges and insights

2.45pm	Alex Buffry	Use of CU&RUN to identify novel interactions in *Drosophila* eye development
3pm	Ariane Ramaekers	EyeHex toolbox for complete segmentation of ommatidia in fruit fly eyes
3.04pm	Mathew Quinn	The *Anopheles gambiae* larval visual system
3.08pm		*Coffee*
3.40pm	Amel Chtioui	Dissecting intraspecific variation in compound eye size in *Drosophila melanogaster*
3.44pm	Maike Kittelmann	*Drosophila* vision - Investigating the developmental basis of eye size variation and thermal plasticity
3.48pm	Amber Harper	The initiation of eye development in the spider *Parasteatoda tepidariorum*
3.52pm	Gordon Wiegleb	Divergence of head morphology in closely related *Drosophila* species on single-nuclei resolution
4.07		*Conclusions*

Abstracts – Keynotes and Contributed Talks

Monday 20th March

2pm Keynote talk: Development, functions and evolution of turbanate eyes of mayflies

Isabel Almudi

tba

2.30pm Evolution of red receptors in nymphalid butterflies

Gregor Belušič

Butterflies have excellent colour vision and are often able to discriminate hues in the red part of the spectrum. This ability is due to the photoreceptors with peak sensitivity beyond 580 nm. Evidence for these »red« receptors has been missing in the largest butterfly family, the brush-footed butterflies (Nymphalidae). Using single cell recordings, optical and anatomical measurements, we have recently discovered that the retina in many nymphalids has red-sensitive photoreceptors. In most cases, these are the basal cells R9 in the red-shining ommatidia, which contain a red filtering pigment and where the cells R1&2 express a LW, green-sensitive opsin, instead or in addition to the blue opsin. These green-sensitive R1&2 are postsynaptic to the red-sensitive R9 and together form a colour opponent pair. Across the nymphalid phylogeny, the red-green opponent pair has been randomly switched on demand. In the tribe Melitaeini, the red receptors have evolved independently and are allocated to the cells R3-8. In the tribes Argynnini and most of Melitaeini, the red receptors are found only in males. The eyes of these butterflies are often highly regionalized, with a dorso-ventral gradient of spectral receptors or a sharply delineated, red-shining ventral zone. The complex phylogenetic patterns of retinal organization in Nymphalidae demonstrate the remarkable evolutionary flexibility of butterfly eyes.

2.45pm Many modes of opsin evolution in Lepidoptera

Peter Mulhair

Colour vision in insects is determined by signalling cascades, central to which are opsin proteins, resulting in sensitivity to light at different wavelengths. In certain groups, lineage specific evolution of opsin genes, in terms of copy number, shifts in expression patterns and functional amino acid changes, has resulted in changes in colour vision and subsequent behavioural and niche adaptations. Lepidoptera are a fascinating model to address whether variation in opsin content and sequence evolution correlates with changes in vision phenotype. Until recently, the lack of high quality genome data representing broad sampling across the lepidopteran tree of life has greatly limited our ability to accurately study this question. Here, we annotate opsin genes from 219 lepidopteran genomes representing 25 families, reconstruct their evolutionary history and analyse shifts in selective pressures between genes and species. We discover more than 40 duplication events in LWS, Blue and c-opsin genes across ~300 million years of lepidopteran evolution. While many duplication events are species or family specific, we find retention of an ancient LWS duplication derived by retrotransposition in the speciose superfamily Noctuoidea (families Nolidae, Erebidae and Noctuidae). This conserved LWS retrogene shows life stage specific expression suggesting visual sensitivities or other sensory functions specific to the early larval stage. This study provides a comprehensive order-

wide view of opsin evolution across Lepidoptera, showcasing high rates of opsin duplications and changes in expression patterns.

3.00pm Flash talks (Evolution): Atal Pande, Alison Irwin

Coffee break: 3.08pm – 3.30pm

3.40pm Flash talks (Evolution): Stefan Graf, Andre Ampuero-Leon

3.48pm The evolution of eye size in spiders

Lauren Sumner-Rooney

Spider visual systems exhibit striking diversity, supporting a range of behaviours and providing key taxonomic characters. This includes variation to eye number, position, orientation, and size. While all of these hold clues to both the visual ecology and the evolution of the group, eye size reflects the extent and distribution of energetic investment in vision and directly impacts function through limitations on resolution and contrast sensitivity. The distributed nature of the visual system offers an additional degree of freedom for adaptation but has previously complicated the analysis of eye size and ecology. We explored the diversity in eye size and size variation across all spiders in data collected by Wolff et al. (2022) and found that, although the diameter of individual eye pairs is not affected by foraging strategy, ecology can explain some aspects of eye size and its variation across the visual system. To investigate how eye size is determined during development and growth, we studied the expression of key retinal determination genes in developing embryos, as well as the ontogenetic allometry of eyes, in seven different families of spiders with different visual system configuration. We identify several key genes whose expression patterns reflect variation in adult eye size distributions, including eyes absent and Six3.2, but others including atonal do not appear to covary in their spatial expression with eye size. Our results are also consistent with strong selective pressures acting on the allometry of some eye pairs into adulthood, varying both between families and within species, indicating a highly flexible mechanism underpinning optimal eye size.

Break: 4.03pm – 4.15pm

4.15pm Workshop: Predicting vision from morphology

Pablo Currea

Dinner: 7pm

Tuesday 21st March

9.30am Keynote talk: What does a bee see? Imaging the visual world of bees to explore the link between vision and flight

Emily Baird

tba

10am Skylight navigation across insects- evolutionary mechanisms optimizing neural circuit structure and function

Heidi Roth

Most free-living insects rely on a combination of various visual stimuli for navigation, one prominent example being the pattern of polarized. All insect eyes consist of repetitive functional units called ommatidia, whose functionally specialized photoreceptor classes define specific ommatidial subtypes, by being tuned to different kinds of visual information, like brightness, chromatic content, or the angle of polarization. Despite the great morphological diversity of insect eyes, the distribution of ommatidial subtypes (together forming a retinal mosaic for color and skylight polarization vision) shows remarkable similarities across species. In Drosophila, the homeodomain transcription factor Homothorax (Hth) is necessary and sufficient for specifying polarization-sensitive ommatidia in the 'dorsal rim area' (DRA) of the adult eye. Functionally analogous DRA ommatidia for detecting polarized skylight are found at the dorsal margin of most insect eyes analyzed to date. Although homologs of the Hth gene exist across species, it currently remains unknown whether Homothorax plays a role in the retinal pattering across insects. In order to reveal potential mechanisms shaping the skylight polarization circuits of the retinal mosaics across insects, we have identified evolutionarily conserved regulatory sequences within the Hth locus that drive expression in the DRA of flies. Furthermore, we generated antibodies specific for the Hth proteins of honey bees, butterflies, and mosquitos in order to compare pupal eye expression of Hth across these species. All of these species manifest navigational skills and possess specialized regions at the dorsal rim of their adult eyes, meaning we can now test whether the development of their polarization-sensitive detectors shares the same molecular mechanism.

10.15am From molecules to behaviours: insights into opsin-based light perception in echinoderms

Jérôme Delroisse

Echinoderms are marine invertebrates characterized by a pentaradial body plan and a nervous system decentralization. Our research aims to understand how these organisms perceive light via the activation of opsins, which are transmembrane proteins involved in vision and photoreception in metazoans. Our ultimate objective is to reconstruct the evolutionary pathways underlying the diversity of photosensory perception mechanisms and the functionality of associated phenotypes in these invertebrates (e.g., sea stars, brittle stars, crinoids, sea cucumbers).The search for opsin genes has been performed in numerous transcriptomes and genomes giving us an extensive glimpse of the echinoderm opsin diversity. Phylogenetic analyses highlight the presence of many opsin groups within each echinoderm lineage. Globally, most of the ancestral bilaterian-type opsins have been conserved in echinoderms and lost in many other metazoan branches. Within echinoderms, numerous species/clade-specific gene duplications have also been observed. We used immunolocalization methods to identify a variety of photosensory effector organs (i.e., expressing opsins) such as pinnules,

spines, buccal tentacles, and tube feet, but also to pinpoint the potential photosensitivity of the central nervous system (i.e., radial nerves). In vivo approaches highlighted diverse light-mediated behaviours in these organisms, such as visual-like abilities in sea stars, brittle stars, and sea cucumbers; phototaxis in brittle stars and crinoids; ambient light perception needed for the synchronization of feeding activity for brittle stars. Our results highlight the importance of wide-ranging sampling including non-model species to more accurately describe the diversity of genes, sensory organs/cells and associated behaviours involved in light perception in marine invertebrates and better understand the ecological pressures they are facing.

10.30am Flash talks (Function): Lena Stanislawczyk, Pavel Kviatko

Coffee break: 10.38am – 11.10am

11.10am Spectral sensitivity of tortricid moths is not tuned to diel activity period

Alejandro Martín

Leafrollers (Lepidoptera: Tortricidae) are a large family of small moths containing over 10.000 species, many of which are crop pests. It includes both nocturnal and diurnal species and although much is known about their olfactory system due to the importance of pheromones to control them, there is not much information about their visual system. Grapholita molesta, Lobesia botrana and Cydia pomonella are widespread tortricids fruit pests. The adults have discrete periods of sexual activity that occur before, during and after sunset, respectively. We wanted to determine if being active at different times of the day and night is associated with differences in their visual system. Spectral sensitivity (SS) was measured with electroretinograms (ERG) and selective adaptation with green, blue and ultraviolet light. SS curves could be fitted with a triple nomogram template which indicated the existence of three photoreceptor classes peaking at 355 nm, 440 nm and 525 nm. The retinae showed clear regionalization, with fewer blue receptors dorsally. No differences among species were found. Intracellular recordings in Cydia pomonella photoreceptors revealed three photoreceptor classes with narrow sensitivity peaking at 525, 440 and 355 nm. The blue photoreceptors showed opponent responses in the green, which could be altered with current injection. Flicker fusion frequency experiments showed that the response frequency was similar among sexes and species at around 100-120 Hz. Our study sheds some light on the relatively unexplored visual system of this economically important insect group.

11.25 Xport-A functions as a chaperone by stabilizing the first 5 transmembrane domains of Rhodopsin-1

Pedro Domingos

Rhodopsin-1 (Rh1), the main photo-sensitive protein of Drosophila, is a seven transmembrane domain protein, which is inserted co-translationally in the endoplasmic reticulum (ER) membrane. Maturation of Rh1 occurs in the ER, where various chaperones interact with Rh1 to aid in its folding and subsequent transport in the secretory pathway. Xport-A has been shown to be a chaperone/ transport factor for Rh1, but the exact molecular mechanism for Xport-A activity upon Rh1 is not known. Here, mostly based on computational predictions, we propose a model where Xport-A functions as a chaperone in the biosynthesis of Rh1 by stabilizing the first 5 transmembrane domains of Rh1, but not the full length Rh1 protein.

11.40 New lights on ocellar and extraocellar photoreception in sea cucumbers (Holothuroidea, Echinodermata)

Youri Nonclercq

Echinoderms are an intriguing group of deuterostome invertebrates to study the evolution of light perception in metazoans, as they have been known to be sensitive to light despite lacking complex eye structures. Previous studies on sea urchins, sea stars, and brittle stars have identified photoreceptors in various body parts, such as tube feet, spines, and the nervous system. Some studies also lead to the discovery of low-resolution spatial (but eye-less) vision in some species! The light perception abilities of holothurians (aka sea cucumbers), however, have been largely understudied, with some punctual results from ethological studies (e.g., some species fly away from a light source while other species retract their oral tentacles under strong light exposure). To investigate photoreception in sea cucumbers, we used a multidisciplinary approach that focuses on opsins, which are prototypical photoreceptor proteins in bilaterians. Our analysis of genomes and transcriptomes from multiple holothurian species reveals six ancestral opsin types. The expression of rhabdomeric opsin-based extraocular photoreceptors was specifically detected in oral tentacles, radial nerves, and tube feet in the European species Holothuria forskali, suggesting a well-developed extraocular photoreception in these animals. Our investigation also focused on the clade of Apodida, a group of sea cucumbers with elongated bodies and lacking tube feet, which some researchers have suggested have visual structures at the root of their tentacles. Our study demonstrated the expression of a ciliary opsin in the photo-sensory neuroepithelial structures of the tropical species Euapta godeffroyi, which form eyespots at the base of each tentacle. A similar expression of opsins in the baso-tentacular nerves was observed in the small European burrowing species Oestergrenia digitata. Additionally, we detected opsins in the sensory cupules on the inner face of tentacles, which have unknown sensory functions. Finally, our ethological tests on three holothurian species showed that they moved away from (Holothuria forskali and Euapta godeffroyi) or toward (Synapta maculata) a light source, specifically for shorter wavelengths corresponding to blue and green light. Turning the spotlights on these amazing sea cucumbers and more broadly on all echinoderms is important to better understand the mechanisms and evolution of extraocular photoreception in the deuterostome lineage.

11.55 Rhodopsin 7 in Drosophila melanogaster

Pingkalai Senthilan

Rhodopsins are the major photopigments of the fruit fly Drosophila melanogaster. In addition to the six well-characterized rhodopsins (Rh1-Rh6) with different absorption maxima and expression patterns, Drosophila also possesses a seventh rhodopsin (Rh7). Rh7, which forms its own subfamily within the arthropod rhodopsins in phylogenetic trees, is widely distributed among arthropods, although not present in all of them. All Rh7 members have in common that they lack the QAKK motif, which is important for G-protein binding and thus for activation of the signaling cascade. The function, expression pattern, and absorption spectrum of Rh7 are still controversial. Several experiments show that Rh7 is expressed in both the eye and brain of animals, although its expression levels are extremely low compared to other rhodopsins. Behavioral experiments suggest that Rh7 plays an important role in the startle response to sudden lights-off. In general, Rh7 mutants appear to be less active under darkness than control flies. The fact that the startle response also fluctuates strongly throughout the day may indicate an interplay with the circadian clock.

Lunch: 12.10pm – 2pm

2m Keynote talk: Variations on a theme

Fernando Casares

tba

2.30pm Using transcriptomics to identify regulators of the subdivision of early Drosophila eye-antennal imaginal discs: challenges and insights

Lucas Di Pietro N Figueiredo Pinto

In the fruit fly, the adult head derives from a pair of primordia named eye-antennal imaginal discs (EADs) 1. EADs appear in the embryo as small sacs of a few dozens of ectodermal cells located anterior to the brain. They undergo important growth throughout larval development, reaching ~20 thousand cells prior to metamorphosis. During this period, EADs also progressively subdivide into distinct territories, corresponding to the various head sensory and non-sensory tissues2. Recent work demonstrated that genetic changes affecting this subdivision could alter the proportion of distinctly-fated territories, and of the sizes of resulting adult structures, such as eyes and antennae3. Multiple studies investigated the regulation of EAD development, either by genetic screening or more recently using "OMICS" methods4–6. However, most of them focus on processes such as retinal differentiation, which take place during late larval stages (L3 or at the earliest at late L2 stage)4, thus after EADs are already subdivided. As a result, previous attempts to use published data to model the "eye" and "antennal" territory specification failed to capture the subdivision between the two fates (Ramaekers and Thieffry, unpublished). To fill up this gap and shed light onto the regulation of EAD subdivision, we are applying transcriptomic methods – both "in bulk" and on single cells - on early (early L1, late L1 and late L2) EADs, i.e. before, during and after EAD subdivision. We will present our technical approaches to optimize those methods to such small samples. In addition, we recently generated bulk RNA-sequencing data, which successfully captures the transcriptomic profiles of early EADs. We will thus share an overview of these results and of our first analyses.

2.45pm Use of CU&RUN to identify novel interactions in *Drosophila* eye development

Alex Buffry

The compound eyes of insects display remarkable diversity in ommatidia number and size which has significant implications for both vision and behaviour. However, very little is known about the genetic and developmental bases of this diversity. We have identified two closely related species of Drosophila, D. simulans and D. mauritiana, which differ in eye size as a consequence of variation in ommatidia area, rather than ommatidia number. We mapped this difference to a small region on the X chromosome. Further analysis of the expression and functions of genes in this region identified the transcription factor encoding gene, orthodenticle (otd), as a candidate underlying the eye size difference between D. simulans and D. mauritiana. We confirmed otd is required for the correct organisation and size of ommatidia in Drosophila. Furthermore, using ATAC-seq we have identified several regions of open chromatin in eye-antennal disc cells that are candidate enhancers of otd. We are currently testing these regions from D. mauritiana and D. simulans for activity in developing eyes and potentially species-specific expression using reporter genes. The chromatin profile generated from eye-antennal discs also allowed us to predict potential genome-wide direct targets of Otd in developing eyes during L3. To help verify these candidate Otd targets, we are performing CUT&RUN on eye discs with an endogenously tagged otd allele. Our identification of the contribution of otd to natural variation in eye size between closely related species provides important insights into the mechanisms underlying the development and evolution of compound eye size in insects.

3pm Flash talks (Development): Ariane Ramaekers, Matthew Quinn

Coffee: 3.08pm – 3.40pm

3.40pm Flash talks (Development): Amel Chtioui, Maike Kittelmann, Amber Haper

3.52pm Divergence of head morphology in closely related Drosophila species on single-nuclei resolution

Gordon Wiegleb

Morphological diversification facilitates adaptation to changing environments. The genetic basis of natural variation of morphology remains largely elusive. The adult head of *Drosophila* develops from the eye-antennal imaginal disc (EAD). During larval development, the cells of the EAD differentiate and give rise to the compound eyes, the ocelli, maxillae, the antennae, and the head capsule. Comparative morphological and genomic approaches have been successfully applied to study the genetic basis of inter- and intraspecific differences in head morphology. However, the developmental dynamics of cell populations in head development are highly complex over the course of larval development and not much is known about cell-type specific changes in gene expression and interspecific differences in relative cell type proportions that potentially influence adult head morphology.

To gain more detailed insight into these processes, we are applied single-nucleus RNA-seq (snRNA-seq) to gain in-depth insights into gene expression dynamics throughout EAD development in the three *Drosophila* species *D. melanogaster, D. mauritiana* and *D. simulans* at five developmental time points on the level of cell populations. The time points were chosen to cover major developmental events from growth and proliferation to differentiation. We confirm that our data captures major processes and cell types of EAD development. We employ our data to distinguish species- and cell population-specific gene expression signals. This data provides a resource to investigate gene expression divergence, as well as to identify differences in relative cell population compositions between the species.

4.07pm Close

Abstracts - Flash talks

Visual Allometry in Earwigs (Dermaptera)
Atal Pande

Compound eyes are the dominant eye type among arthropods and offer many adaptive possibilities. Due to their design, there is an inherent trade-off between resolution, contrast sensitivity, and field of view. However, how this constraint varies with eye size remains largely unknown. Earwigs (Dermaptera) are a small order of polyneopteran insects that usually inhabit riparian habitats but occupy a number of niches and display a wide variety of feeding habits. Despite being mostly nocturnal, earwigs possess apposition compound eyes, in contrast to the superposition eyes of many nocturnal insects. This may have implications for their visual adaptations. Here, we show that earwigs with bigger eyes have greater sensitivity as well as finer resolution, while maintaining a constant field of view. We used micro-computed tomography (micro-CT) to calculate ommatidial diameters, interommatidial angles, eye surface areas, and the size and direction of the visual field in 109 species across the phylogeny of earwigs. An increase in eye surface area correlates with increased facet sizes and facet number, whereas the interommatidial angle decreases, all with slightly negative allometries with respect to head size (measured as centroid size). Additionally, we show that earwigs with larger body lengths have larger heads but proportionally smaller eyes. Our results demonstrate that earwigs with larger eyes invest primarily in increasing resolution by decreasing the angles between the facets, enlarging the individual facets, and increasing overall facet number. The enlargement of facets also brings a slight predicted increase in sensitivity. It is likely that increasing sensitivity as well as resolution helps these mainly nocturnal insects to navigate in the dark, whereas their concealed habitats in cracks and crevices likely explain the apparently lesser need to increase the field of view.

Evolution of large eyes in the conch snail group Stromboidea

Alison Irwin

The marine gastropod group Stromboidea comprises two clades of greatly contrasting eye sizes, providing an excellent opportunity for eye evolution studies. Visual systems in the group vary from small eyes at the base of cephalic tentacles to large eyes on the ends of long eyestalks with fine spatial resolution vision. To better understand the evolution of large eye size, we examine relationships between ecological factors and a series of morphological traits within 56 species across the superfamily. We use Sanger sequencing data for four genes to build a robust phylogenetic framework of the group, including fossil time-calibrations, onto which we map depth ranges and morphological measurements obtained from rapid µCT-scanning. Ancestral state reconstructions suggest that extremely large eye size evolved at least twice within the clade containing all large-eyed families. We also found that maximum depth is correlated with absolute eye size, with stromboids groups that inhabit bright, shallow-water environments possessing larger eyes. Compared to small-eyed xenophorids, which occupy a wide range of depths and extend into much deeper waters, members of the shallow-water family Strombidae also have bigger eyes than would be expected for their body size, suggesting that vision is important in behavioural tasks.

Comparative morphology of modified ocelli in apoid wasps
Stefan Graf

Most flying insects have not only two large compound eyes, but also three single-lens eyes on the vertex. These ocelli were thought to work together as a simple light sensitivity sensor, but modern studies have shown their involvement in flight control and navigation. Thus, it is surprising that apoid wasps developed strong modifications to this vital organ, including dramatic changes to shape and size. Comparable modifications are otherwise unknown in flying insects, but developed multiple times

independently within apoid wasps. We investigated the internal morphology and possible functions of modified ocelli in apoid wasps, which, while of great taxonomic interest, remained enigmatic. Modifications range from slight elliptic deformations to C- or comma-shaped lenses. While these are often described as “scars” and “reductions” in the literature, our study is the first to show that, despite these strong modifications, the ocelli may still be functional. According to our preliminary data, they might have maintained the morphological properties to recognize polarisation patterns in skylight and likely a bipartite retina, potentially facilitating the formation of somewhat sharp images. We hope to shed further light on their morphology and possible function by combining µCT and synchrotron scans with classical histology to obtain light and electron microscopy pictures.

To see beyond the shell: Sensory organs in chitons (Mollusca: Polyplacophora)

Andre Ampuero-Leon

tba

ts-CRISPR-induced knockdown of Rab proteins leads to defects in TRPL transport in Drosophila photoreceptor cells.

Lena Stanislawczyk

Protein aggregation and protein accumulation, triggered by defects in intracellular protein transport mechanisms, are associated with various human neurodegenerative diseases. An in vivo model system for intracellular transport of membrane proteins is the light dependent translocation of the TRPL (transient receptor potential like)-ion channel in Drosophila photoreceptor cells. While TRPL is located in the rhabdomeric membrane in dark adapted flies, it is transported into the cell body upon light exposure. With further dark adaptation, TRPL is recycled back to the rhabdomeric membrane. A method to identify novel transport-associated proteins is the genetic mutagenesis screen system ts-CRISPR-Cas 9 (tissue-specific-Clustered Regularly Interspaced Short Palindromic Repeats-Cas9). Prior to the analysis of potential translocation components of TRPL, the efficiency of the ey-Gal4-UAS-uS-Cas9 driver line was tested. It was crossed with flies expressing sgRNA-norpA. Western Blot analysis revealed an efficient knock down of the norpA gene product PLCβ. Potential candidates that may be involved in TRPL translocation comprise Rab proteins. Rab proteins are small guanosine triphosphatases that regulate protein transport along endocytic and exocytic pathways and are involved in vesicle budding, membrane fusion, and interactions with the cytoskeleton. To identify specific Rab proteins associated with TRPL translocation, different Rab proteins were knocked-down, using specific sgRNAs. The effects on TRPL translocation were examined via water immersion microscopy and immunohistochemistry. Our findings show that RabX2 and Rab3 have an effect on TRPL recycling back to the rhabdomeric membrane upon dark adaption. Rab10, however, seems to play a role in TRPL internalization.

Insect retinal muscles in the context of visual ecology

Pavel Kviatko

Insects are often thought to have eyes that cannot move independently of the head. However, previous research has shown that true flies have muscles that can shift the whole retina, effectively granting them eye movements [1-4]. Recently, these retinal movements have been functionally characterized in Drosophila [5]. It is still unclear, however, how prevalent retinal movements are among insects, and whether they have evolved in the context of specific visual adaptations. In our work we aim to answer two questions: 1) Is there a link between the visual anatomy and presence of retinal movements across the insect orders? 2) Are there correlations between the visual ecology and the features of the retinal movements and muscles across Diptera? To do this, we are using confocal laser-scanning microscopy to search for putative retinal muscles in sliced and whole

insect heads stained for actin and chitin. Across the Diptera species examined so far, we have found two retinal muscles per eye, similar to the muscles described in Muscomorpha: One muscle, musculus orbito-scapalis (MOS) originates from the antero-dorsal region of the medial rim of the circumocular ridge and inserts at the scapus of the antenna. A second muscle, musculus orbito-tentoralis (MOT) originates at the antero-ventral region of the medial rim of the circumocular ridge and inserts at the tentorial bridge. We have just started to investigate other insect orders and while our limited observations do not allow for a correlative inference yet, they already suggest an even richer world of active insect vision. We will extend our search for putative retinal muscles into more insect taxa, including species with bigger head size using light sheet fluorescence microscopy and micro-computed tomography. Recordings of retinal movements in live tethered insects will allow us to confirm the function of putative retinal muscles.

1. Burtt, E. T. & Patterson, J. A. Internal Muscle in the Eye of an Insect. Nature 228, 183–184 (1970).
2. Hengstenberg, R. Das augenmuskelsystem der stubenfliege musca domestica: I. Analyse der „clock-spikes" und ihrer Quellen. Kybernetik 9, 56–77 (1971).
3. Patterson, J. The Eye Muscle of Calliphora vomitoria L. J. Exp. Biol. 58, 565–583 (1973).
4. Franceschini, N., Chagneux, R., Kirschfeld, K. & Muecke, A. in Göttingen Neurobiology Report (eds. Elsner, N. & Penzlin, H.) Vol. 1, 275 (Thieme, 1991).
5. Fenk, L. M. et al. Muscles that move the retina augment compound eye vision in Drosophila. Nature (2022).

EyeHex toolbox for complete segmentation of ommatidia in fruit fly eyes

Ariane Ramaekers

Variation in insect compound eye size is investigated from various perspectives, ranging from evolutionary, functional, developmental and biomedical. Large-scale studies of eye size variation would benefit from automated measures of features such as ommatidia number, size or organization. However, mostly due to their prominent curvature, the segmentation of compound eyes is far from being trivial. In this flashtalk, I will present the EyeHex toolbox for complete segmentation of ommatidia in fruit fly compound eyes. This toolbox makes use of the highly ordered ommatidia positioning in a hexagonal grid to determine the ommatidia location and number accurately. EyeHex combines the Trainable Weka Segmentation (TWS)1 machine learning module with a MATLAB-based user interface. We successfully applied EyeHex on various compound eye image types – acquired using a variety of imaging devices – SEM, macroscope, stereomicroscope, brightfield microscope. Therefore, our tool may constitute a simple, accurate and cost-effective method to automatically segment ommatidia.

The Anopheles gambiae larval visual system

Matthew Quinn

Stemmata are larval eyes found uniquely in Holometabolous insects such as flies, butterflies and beetles (Friedrich, 2003). While the stemmata have been well studied in other insects, their functional role, morphology and fate during metamorphosis is not well studied in mosquitoes. Work on Aedes aegypti mosquitoes indicated that larvae possess both the larval and the developing adult eyes, and both are functional in the later stages of larval development (Mysore et al., 2014). Opsins are G-protein coupled receptors that determine the sensitivity of an insect to different wavelengths of light (Brody & Cravchik, 2000). Larval and adult eyes are predicted to express different types of opsin genes in Aedes aegypti (Rocha et al., 2015). However, this has not been proven in Anopheles gambiae. Previous studies in Anopheles gambiae found that opsin 6 is more highly expressed during the larval

stage, while opsins 1 and 3 dominated the expression profile in the adult stage (Jenkins & Muskavitch, 2015). However, this study only looked at 3 stages of the mosquito life cycle: L1, L3, and adult. The detailed opsin expression and functionality at different life stages thus remains unexplored. Eye development and the reduction in size of the larval eye has been studied to a greater extent in other holometabolous insects. In Drosophila melanogaster, of the 12 photoreceptors found in the larval eye, only 4 survive metamorphosis as they dedifferentiate and are recycled into the developing adult eye during pupation (Helfrich-Förster et al., 2002). In three species of caddisflies the larval eye is reduced in size, remains connected to the optic lobes in the back of the adult eye but it is unknown if it is still functional (Hagberg, 1986). In beetles, the larval eye is reduced and is situated between the medulla and lobulla where the rhabdoms remain organized but any functional role is enigmatic (Friedrich, 2003; Friedrich et al., 1996, 2006). In butterflies the larval eye is reduced to pigmented cells within the optic lobes (Sbita et al., 2007). The larval eye has also been shown to persist 48 hours after pupation in Aedes aegypti mosquitoes (Mysore et al., 2014). It is reasonable to conclude that a similar eye development strategy may occur in Anopheles gambiae, but this has not yet been studied. We thus aim to characterize expression of opsin genes throughout all stages of development, analyse anatomy of the larval visual system and investigate larval behavioural responses to chromatic stimuli. The life cycle of mosquitoes is made up four typical stages: egg, larva, pupa and adult. We used qPCR to quantify opsin genes over 13 days in the mosquito life cycle. We assayed egg stage (day 0 – 1), first instar (d1), second instar (d2), third instar (d3-5), fourth instar (d 6-7), pupae (d8 – 9), emerging adult (d 10 – 12), and sexually mature adult 7 days post emergence. Our results show that opsin 6 (op6), a long wavelength opsin, has significantly higher expression than all other opsin in days 1 – 4. The pattern of opsin expression is more variable from day 5 to pupation. In contrast, opsins 1 (op1) and 3 (op3) are dominating the expression profile in the first day of the adult stage. This is similar to results found by Jenkins & Muskavitch (2015). We demonstrate that genes op1, op3, op8 and op9 all significantly increase in expression in adult males (d7). This is similar to what has been shown in Aedes albopictus, where Aaop1, and Aaop2, both orthologues to op1 and op3 in Anopheles gambiae respectively, increased in expression from days 1 – 7 (Liu et al., 2022). Interestingly, we did not find the same trend in females, where only op9 increased in expression. This may be because females were blood-fed 3 days before homogenization, and this will be explored in further analyses by qPCR of non-blood-fed day 7 females. op6 slightly decreased in expression in both male and female adult Day 1 – 7 adult stages, although the effect was not significant. The decrease in op6 expression followed by the subsequent increase in opsins that characterize the developing adult eye in other species could be the first evidence of the degenerating larval photoreceptors in Anopheles gambiae. To investigate the anatomy of the visual system, we collected larvae and pupae on days 1, 2, 8, and 13 after hatching from the embryo. We acquired synchrotron data to visualise the anatomy by creating 3D models of the larval visual system for the first time. We show that not only Anopheles gambiae possess both the larval and adult eye that are present at the same time later in development, but the innervation of both eyes forms a single optic nerve as previously seen in Aedes aegypti larvae (Mysore et al., 2014). Morphological structures such as the optic lobes and optic nerve also increase in volume through development. We demonstrate that while the adult eye increase in ommatidia number, the larval eye decreases in size as it transitions from larva to pupa. In conclusion, we show that op6 is more highly expressed than all other opsins during the early larval stage. Simultaneously opsin op1 and op3 increase in expression later in development. In line with the decrease of op6, we show that the size of the larval eye decreases in both volume and surface area in pupation. The degeneration of the larval eye is the first evidence of this process in Anopheles gambiae as it transitions to the adult visual system.

Dissecting intraspecific variation in compound eye size in Drosophila elanogaster

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Understanding the evolution of complex morphological traits is still one of the main challenges in evolutionary biology. This requires the identification of the causative genomic regions and the molecular changes responsible for phenotypic differences. Natural variation in compound eye size is pervasive in insects. To study eye size variation, Drosophila melanogaster is a great model to reveal the developmental and molecular mechanisms underlying complex trait evolution using a micro-evo-devo approach. In this study, we employ the Drosophila melanogaster Genetic Reference Panel (DGRP) to genetically dissect the intraspecific variation in eye size. A tremendous variation in ommatidia number between 162 DGRP strains was observed. To establish a link between phenotypic divergence and genetic changes, we performed genome wide association studies (GWAS). A functional validation screen of the three genes containing most associated single nucleotide polymorphisms (mbl,trim9, CG15498) confirmed a potential involvement in eye size regulation. To better understand the cellular and developmental processes regulated by these genes, we analyzed trim9 in more detail. This gene codes for a RING domain E3 ubiquitin ligase. Knockdown of this gene in the eye-antennal imaginal disc (EAD) resulted in an increase of apoptotic cells and a severe reduction or the entire absence of compound eyes. Our data suggests that trim9 may be a negative regulator of apoptosis during eye development. The role of apoptosis in ommatidia number variation in Drosophila has not been observed yet, making this study an exciting start for upcoming research on morphological evolution. In ongoing experiments, we test, whether variation in trim9 expression and thus differences in apoptosis underlies natural variation in eye size among DGRP lines.

Drosophila vision - Investigating the developmental basis of eye size variation and thermal plasticity.

Maike Kittelmann

The striking diversity in eye shape and size among insects reflects adaptations to different habitats and behaviours. Eye size directly impacts the quality of vision, but how eye size is specified during development and how environmental variation is integrated into this process is not well understood. To analyse this further, we examined eye morphological parameters as well as the impact of temperature on eye size across multiple strains of Drosophila melanogaster, Drosophila mauritiana and Drosophila simulans. While eye size tends to be largest in D. mauritiana and smallest in D. melanogaster, we found that both ommatidia number and size vary greatly between strains across all three species. We then modelled the impact of differences in eye morphology on vision using 3D ultrastructural information from synchrotron radiation microtomography, and tested hteir vision in vivo using behavioural assays. Intriguingly we have also found that the impact of environmental factors like temperature on eye size varies within species. While some strains of D. mauritiana and D. melanogaster maintain eye size across temperatures others show thermal plasticity as observed for other body parts like wings. This suggests differential effects of temperature on the regulation of eye developmental processes like growth, cell proliferation and differentiation between strains. Further morphological, developmental, and behavioural analyses of Drosophila with varying eye thermal plasticity will provide valuable insights into mechanisms that specify eye size and therefore affect vision.

The initiation of eye development in the spider *Parasteatoda tepidariorum*

Amber Haper

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