The Developmental Plasticity of Fruit Fly Vision

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

THE DEVELOPMENTAL PLASTICITY OF FRUIT FLY VISION

A dissertation submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PSYCHOLOGY

by

John P. Currea

2021
To: Dean Michael R. Heithaus  
College of Arts, Sciences and Education

This dissertation, written by John P. Currea, and entitled The Developmental Plasticity of Fruit Fly Vision, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Florida International University, 2021
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DEDICATION

To Emily’s smile.
ACKNOWLEDGMENTS

I would like to thank my co-mentors and the members of my graduate committee for guiding me along this bizarre and intriguing process. I’m especially grateful to Dr. Jamie Theobald for his frequent help and inspiring (sometimes beer-fueled) discussions, and to Dr. Robert Lickliter for his guidance, support, and philosophical inquiries. And to my wife Emily for having my back and tolerating my scientific ramblings. A special thank you to the past and present members of the Theobald and Lickliter labs who helped me throughout graduate school, especially Dr. Carlos Ruiz, Dr. Starlie Belnap, Abdullah Ahmand, Yash Sondhi, Dr. Nicolas Palermo, Dr. Ravindra Palavalli-Nettimi, Elina Barredo and Dr. Joshua Smith for their invaluable contributions to my work and worldview.

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ABSTRACT OF THE DISSERTATION
THE DEVELOPMENTAL PLASTICITY OF FRUIT FLY VISION
by
John P. Currea
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Miami, Florida
Professor Jamie Theobald, Co-Major Professor
Professor Robert Lickliter, Co-Major Professor
In this dissertation we explore the morphological and neural plasticity underlying vision at different scales—within and between species of Drosophila—to elucidate the role of eye development in the evolution of vision. In chapter 2, we offer a tool to accelerate large-scale research into compound eye morphology, and validate it on the eyes of several insect orders and image media. Then, in chapter 3 we demonstrate the developmental plasticity of eye morphology and neural summation in fruit flies, finding an interesting interplay between the two systems. In chapter 4, we elucidate the role of visual plasticity and neural summation in the evolution of vision by comparing vinegar and desert flies. Finally, in chapter 5, we show how future and ongoing work is diving into the mechanisms underlying this visual plasticity by measuring the effect of early temperature, exploring regional differences across the visual field, and detecting light-induced circadian activity.
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PREFACE

Chapter III was published in the journal *Vision Research* and Chapter II was submitted for publication in the journal *Communications: Biology* and received a response to revise and resubmit. Both the full manuscript of Chapter III and the preprint of Chapter II are open access (on PubMed Central and bioRxiv, respectively) so they can be reproduced freely here for non-commercial purposes with the citations provided below. Chapter IV is in review at *iScience*.

CHAPTER II


CHAPTER III

CHAPTER I

INTRODUCTION
Nearly all animals that can see move their eyes in response to coherent motion that occupies a large portion of the visual field, called wide-field motion. Many (including humans) respond by moving their eyes independently from the head, the *optokinetic response*, or by moving their whole body or head, the *optomotor response* (Land 2019; Land and Nilsson 2012). These visuomotor responses are fast—20 to 40 ms in fruit flies—and both stabilize background motion (Land 2019) and provide estimates of self motion in 3D to correct for course perturbations (Theobald, Ringach, and Frye 2010). Optokineti

Optokinetic and optomotor responses incorporate wide-field visual motion detection, which integrates the output of many elementary motion detectors (EMDs) with receptive fields distributed across the visual field. EMDs estimate local motion velocities by comparing the outputs of pairs of nearby photoreceptors, following the same fundamental algorithm across both vertebrate and invertebrate eyes. Specific sets of EMD outputs are combined downstream, culminating in the computation of wide-field motion. (Borst and Egelhaaf 1989; van Santen and Sperling 1985).

The algorithm underlying EMDs was discovered through behavioral experiments of the optomotor response in weevils more than 60 years ago (Hassenstein and Reichardt 1956). Since then, the neural implementation of the EMDs and their wide-field integration have been substantially elucidated in the visual system of flies, providing one of the best understood perceptual neural systems (Theobald 2018). In flies, the components of individual EMD circuits are distributed in parallel across three of the four optic neuropils (the lamina, medulla, and lobula) and, though their implementation is more complicated than the original Hassenstein-Reichardt correlator model, elaborated models predict behavioral responses well (Theobald 2018; Tuthill, Chiappe, and Reiser 2011). In flies,
specific subsets of EMDs are processed downstream by tangential cells in the last remaining neuropil, the lobula plate (~20 studied so far). Each lobula plate tangential cell (LPTC) corresponds to an optic flow field of a specific 3D rotation or translation that collectively form a retinotopic map of their motion in 3D space (Borst and Weber 2011). Insects have compound eyes, which are composed of many facets (ommatidia), each of which corresponds to roughly a pixel of the visual field, and one of the two inputs of an EMD. Each ommatidium is equipped with a lens, size-limited by the discrete nature of light absorption and the diffraction of light through narrow apertures (for more information, see Chapters 2–4). As a result, compound eyes have low spatial resolution, meaning they are generally poor at detecting spatial detail compared to camera-type eyes, which have a single lens. Because of this geometric relation, for a compound eye to achieve the spatial resolution of the human camera-type eye, it would have to be a sphere at least 1 meter wide (Kirschfeld 1976; Land and Nilsson 2012)! However, contrary to common misconception, a compound eye forms a single coherent, retinotopic image downstream. Along with EMDs and LPTCs, fly optics and retinal physiology are very well understood (Juusola et al. 2017; Juusola and Hardie 2001; Juusola and Song 2017; Ready, Hanson, and Benzer 1976; Snyder, Stavenga, and Laughlin 1977) and have been used to make substantial progress in the study of visual ecology through comparisons between species (Cronin et al. 2014; Land and Nilsson 2012; Theobald, Warrant, and O’Carroll 2010).

One of the most time-consuming jobs in characterizing the visual capacity of compound eyes is to measure and count by hand the ommatidia in micrographs. Throughout the process of counting hundreds of ommatidia in hundreds of eye images, we developed a
method for automating ommatidia detection in images, which we describe and validate in chapter 2. Due to the ubiquity and diversity of arthropods, compound eyes are the most common image-forming structures on earth, and contribute to research in vision, evolution, development, plasticity, neurobiology, and neuroethology. Though anatomical measurements cannot fully replace meticulous optical techniques, the two computer programs we offer in chapter 2 enable accelerated large-scale research into the diversity of compound eye morphologies especially using micro-CT data. We validate these methods on a wide range of eye shapes and sizes by comparing their output to manual measurements and those established in the literature, and show how they minimize the substantial labor required to study vision in arthropods.

Many components of the optomotor response are developmentally plastic to properties of the early environment. Visually-guided behavior in fruit flies, including courtship and mate choice, are developmentally plastic to early ambient light levels (Hirsch and Tompkins 1994). Chapters 3 and 4 of this manuscript show how the optics and structure of the eye, the detection of motion—implicating EMD function—and thus optomotor behavior are developmentally plastic within fruit flies. Also, individual fruit fly photoreceptor function (Wolfram and Juusola 2004) and volume as well as lamina, medulla, and lobula plate neuropil volumes are affected by early adult ambient light levels (Barth et al. 1997). So, many components of wide-field motion perception and the optomotor response vary within a species in response to environmental or experiential factors. However, this has been largely overlooked due to conventions of animal husbandry that lead to artificially uniform distributions, and so we know little about the developmental plasticity of vision in flies.
In particular, the growth of all insects that undergo a full metamorphosis (those with proper larval and pupal stages, Endopterygota)—including fruit flies—is plastic to features of the early environment during larval development, such as nutrition and temperature (David, Moreteau, and Gauthier 1994; Davidowitz and Nijhout 2004; Shingleton, Mirth, and Bates 2008). When available food is low or larval density is high, larvae that survive through the terminal growth period (TGP) forgo continued growth, pupariating sooner and emerging as smaller but otherwise healthy adults that are typical in nature. These adults possess even smaller eyes than the already typically small fruit fly eyes, and therefore face more severe physical limitations of diffraction and shot noise due to light passing through small and narrow optics. How do even smaller conspecifics cope with inferior optical quality and compete with their larger peers for resources and mates? This question was largely unexplored because in a lab, fly larvae are conventionally provided abundant food, resulting in large and uniform adult sizes. In chapter 3, we address this question by removing larvae from the substrate during the TGP, generating a size distribution akin to natural populations. Ultimately, we characterize the effect of body size on eye morphology and visual responses, and find that the neural development underlying the optomotor response—specifically the use of neural temporal summation—is also plastic, allowing the visual system to adjust to the plasticity of eye morphology.

Finally, the developmental trade-offs in vision made by different species—favoring spatial acuity, temporal acuity, or contrast sensitivity as eye size decreases—reflect differences in natural history. In particular, two species with very different visual environments and ecologies should likely demonstrate differences in visual development,
especially given the high energy demands of seeing and flying. So, in chapter 4 we compare the visual allometry and performance of the vinegar fly (*Drosophila melanogaster*) to another *Drosophila* species with a different habitat and ecology, a cactophillic desert fly from the southwest deserts of North America (*Drosophila mojavensis*). We find that desert fly vision is suited to the flat, bright, and barren characteristics of the desert in several ways. These visual differences enable the desert fly to navigate in the harsh desert and yet accommodate their crepuscular and biphasic adult lifestyle, while enabling the vinegar fly to see under wide-ranging light levels around the world.

In this thesis I determine the developmental effects of habitat on adult fly vision within a species (Chapter 3), the evolutionary effects of habitat between species (Chapter 4), and present a robust method to automate future studies characterizing the visual capacity of compound eyes (Chapter 2). The proceeding chapters therefore explore the morphological and neural plasticity underlying vision at different scales—within and between species of *Drosophila*—to elucidate the role of eye development in the evolution of vision.
References


CHAPTER II
MEASURING COMPOUND EYE OPTICS WITH MICROSCOPE AND MICROCT IMAGES

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The arthropod compound eye is the most prevalent eye type in the animal kingdom, with an impressive range of shapes and sizes. Studying its natural range of morphologies provides insight into visual ecology, development, and evolution. In contrast to the camera-type eyes we possess, external structures of compound eyes often reveal resolution, sensitivity, and field of view if the eye is spherical. Non-spherical eyes, however, require measuring internal structures using imaging technology like MicroCT (μCT). μCT is a burgeoning 3D X-Ray imaging technique that has been used to image arthropod muscles, brains, ocelli, and eyes. Thus far, there is no efficient tool to automate characterizing compound eye optics in either 2D or 3D data. We present two open-source programs: (1) the ommatidia detecting algorithm (ODA), which automatically measures ommatidia count and diameter in 2D images, and (2) a μCT pipeline (ODA-3D), which calculates anatomical acuity, sensitivity, and field of view across the eye by applying the ODA to 3D data. We validate these algorithms on images, images of replicas, and μCT scans from eyes of ants, fruit flies, moths, and a bee.
There are about 1.3 million described arthropod species, representing about 80% of
described animal species (Zhang 2013). They range vastly in size, lifestyle, and habitat,
with body lengths from the 85 µm ectoparasitic crustacean *Tantulacus dieteri* (Mohrbeck,
Arbizu, and Glatzel 2010) to the 0.5 m green rock lobster, *Sagmariasus verreauxi*
(Holthuis 1991; Kensler 1967), and occupying nearly every ecological niche. Likewise,
arthropods wield an array of eye architectures, most commonly the compound eye
(Cronin et al. 2014; Land and Nilsson 2012). Recent progress understanding compound eye
development facilitates studying the selective pressures on the development of
compound eyes (Casares and McGregor 2020; Currea, Smith, and Theobald 2018;
Friedrich 2003; Gaspar et al. 2019; Harzsch and Hafner 2006). Arthropod compound eyes
are therefore a prime subject in the study of vision (Cronin et al. 2014; Land and Nilsson
2012): they are the most prevalent type in the animal kingdom, and include a variety of
morphologies that emerge from relatively well-understood development.

Eye morphology is fundamental to how animals see because structure physically limits visual capacity (Land and Nilsson 2012). Depending on light level and image motion,
some optimal eye architecture will maximize the ability to differentiate brightness levels
and resolve spatial details (Land 1997; Snyder, Laughlin, and Stavenga 1977; Snyder,
Stavenga, and Laughlin 1977). The critical role of morphology implies that natural compound eye diversity will provide insight into visual ecology, development, and evolution. Here we offer two open-source programs written in Python to characterize compound eyes: (1) the ommatidia detecting algorithm (ODA), which automatically detects the individual facets, (called ommatidia), in 2D images, and (2) a multi-stage µCT
pipeline (ODA-3D) which applies the ODA to segment individual crystalline cones and characterize the visual field.

In contrast to the camera-type eyes we possess, many structures that limit optical quality in compound eyes are externally visible. A compound eye is composed of many individual ommatidia, each of which has a lens and crystalline cone that direct light onto photoreceptors. Ommatidia usually represent the maximal individual pixels of the transduced image, so their number limits the total number of images an eye can form, or its spatial information capacity, and they can be counted in microscope images. Their count ranges from ~20 ommatidia in the eye of one of the smallest flying insects, the fairyfly *Kikiki huna* (body length=158µm; Huber and Beardsley 2000; Huber and Noyes 2013) to >30,000 in large dragonflies, which have enhanced vision for hunting prey (Cronin et al. 2014).

In each ommatidium, the lens size restricts aperture, which limits the rate of light collection. This becomes crucially important when photons are limited, such as in dim light, or during fast image motion (Snyder, Laughlin, et al. 1977; Snyder, Stavenga, et al. 1977; Theobald 2017; Warrant 1999). Compound eyes divide into two structural groups which collect light differently. Apposition eyes separate light with pigment cells between ommatidia, and restrict it to a single rhabdom such that lens size directly limits optical sensitivity (Figure 1A). Superposition eyes direct light through a clear zone, so many facets can contribute to each point in an image (Figure 1B), thereby multiplying final sensitivity (although the image remains subject to the diffraction limit of individual aperture size).
Ommatidia count and diameter can be derived from 2D images. Although several algorithms and software plugins have been proposed to extract ommatidia, they require user input for each image, and underestimate ommatidia counts (Woodman, Todd, and Staveley 2011), overestimate ommatidial diameter (Schramm et al. 2015), or were not validated against manual measurements or measurements in the literature (Diez-Hermano et al. 2015; Iyer et al. 2016; Vanhoutte, Michielsen, and Stavenga 2003). In addition, none are tested on multiple species, over a substantial range of eye sizes, or with different media. To support this effort, we offer an open-source program written in Python called the ODA. We test the reliability and validity of this technique on single images of 6 eye molds of 5 different ant species ranging dramatically in size, microscope images of 39 fruit flies (Drosophila melanogaster), and processed images of MicroCT (µCT) scans of 2 moths (Manduca sexta and Deilephila elpenor) and one bee (Bombus terrestris). The method is widely accessible because it only requires microscope imaging.

For spherical eyes, the lens diameter measurements provided by the ODA can be combined with measurements of eye radius (using the luminous pseudopupil technique, for instance) to measure the angular separation of ommatidia, called the interommatidial (IO) angle ($\Delta\phi$). This angle inversely limits spatial acuity or resolution (Land 1997; Snyder, Laughlin, et al. 1977; Snyder, Stavenga, et al. 1977). High spatial acuity affords many behaviors, such as prey, predator, and mate detection, and perceiving small changes in self-motion (Land 1997; Land and Nilsson 2012). For spherical eyes, the IO angle is approximately: $\Delta\phi = D/R$, where $D$ is the ommatidial lens diameter and $R$ is the radius of curvature, assuming the axes of all ommatidia converge to a central point. Fortunately, many compound eyes are safely approximated by the spherical model.
Smaller compound eyes are often spherical and homogenous because photon noise and diffraction constrain IO angle and ommatidial size (Snyder, Stavenga, et al. 1977). Likewise, superposition eyes are often roughly spherical because they optically combine light from many ommatidia (Figure 1B; Land and Nilsson 2012).

But many compound eyes are non-spherical, with ommatidial axes askew to the eye surface. Skewed ommatidia sacrifice sensitivity by reducing the effective aperture as a function of the skewness angle and refraction, but can improve acuity at the expense of field of view (FOV) by pointing more ommatidia onto a small visual field (Figure 1C). Alternatively, skewness can increase FOV at the expense of acuity by spreading a few ommatidia over a large visual field (Figure 1D).

MicroCT (µCT), a 3D X-Ray imaging technique burgeoning in use among comparative morphologists (Baird and Taylor 2017; Buser et al. 2020), is a rich and valuable resource to study arthropod vision. It has been used on arthropods to study muscles (Walker et al. 2014), brains (Smith et al. 2016), ocelli (Taylor et al. 2016), and eyes (Brodrick et al. 2020; Gaspar et al. 2019; Taylor et al. 2018, 2020). Visualizing internal structures in 3D allows calculating visual parameters for non-spherical compound eyes, measuring anatomical IO angles at very high spatial resolution, and segmenting different tissues, such as visual neuropils, within the same dataset.

Our second proposed method, ODA-3D, extracts crystalline cones from a µCT image stack (using the ODA) to measure the size, orientation, and spatial distribution of ommatidia. As mentioned above, we validate it on µCT scans of the spherical eyes of two moth species (M. sexta and D. elpenor) collected by us and an open access scan of a non-spherical, oval bumblebee eye (B. terrestris) used in Taylor et al. (2018). For the
bumblebee, we further demonstrate how ODA-3D can detect oval eye features, measure regional changes in skewness, spatial acuity, and sensitivity, and project onto world-referenced coordinates to more accurately measure FOV.

Compound eyes are the most common image-forming structures on earth. Because there are so many arthropod species and compound eye morphologies, it is challenging but valuable to characterize them in a meaningful, fast manner. Our proposed methods minimize the substantial labor that is typically required in characterizing optical performance in compound eyes and therefore facilitate understanding their role in visual ecology, development, and evolution.

**Methods**

**Specimens**

Microscope images of eye molds or replicas were taken previously on five ant species: *Notoncus ectatommoides* and a Golden tail sugar ant (*Camponotus aeneopilosus*) of the Formicinae subfamily (from Palavalli-Nettimi and Narendra (2018)), a jumper ant (*Myrmecia nigrocincta*) and a bull ant (*M. tarsata*) of the Myrmeciinae subfamily, and *Rhytidoponera inornata* of the Ectatomminae subfamily (from Palavalli-Nettimi et al. (2019)). We further used microscope images of 39 fruit fly eyes (*Drosophila melanogaster*) from Currea et al. (2018).

We obtained micro-computed tomographs (µCTs) of the tobacco hornworm (*Manduca sexta*), the elephant hawkmoth (*Deilephila elpenor*), and the bumblebee (*Bombus terrestris*). Vouchered moth specimens from the Florida Natural History Museum were stored at -20°C in 95% ethanol, then heads were sliced, with antennae removed, and soaked in staining solution (I2+KI, equal proportions 1.25% I2 and 2.5% KI solutions) in
Eppendorf vials or falcon tubes for 36–48 hours. *M. sexta* was scanned with a Phoenix V|Tome|X M system with: a 180kv x-ray tube, a diamond-tungsten target, 80 kV tube voltage, 110 µA current, 17.8 mm source object distance, 793 mm object-detector distance, and capture time adjusted to maximize absorption range for each scan. The acquisition consisted of 2300 projections, 8 s each. GE’s datos|x r software version 2.3 processed raw x-ray data, producing voxel size of 4.50074 µm. Volume files were imported into VG StudioMax version 3.3.3 (Volume Graphics, Heidelberg, Germany), eyes isolated with the segmentation tools, then exported as Tiff stacks. *D. elpenor* was scanned with a Zeiss Xradia 520 Versa (Carl Zeiss Microscopy GmbH, Jena, Germany), with: 80 kV tube voltage, 88 µA current, low energy filtering, 22.5 mm source object distance, 210 mm object-detector distance, an indirect detector comprising a scintillator, a 0.392x optical lens, and a camera. The acquisition consisted of 3201 projections, 8 s each, with the adaptive motion correction option in Scout-and-Scan software (Carl Zeiss Microscopy GmbH). The tomographic reconstruction automatically generated a 32-bit txrm set of tomograms with an isotropic voxel size of 3.3250 µm. The XRM controller software (Carl Zeiss Microscopy GmbH) converted data to a stack of 16-bit tiff file. A previously scanned bumblebee eye was used with permission from the authors, available at https://www.morphosource.org/Detail/ProjectDetail/Show/project_id/646. This sample was scanned with a synchrotron x-ray source, (Baird and Taylor 2017), see Taylor et al., (2018) for the details.

**Ommatidia Detecting Algorithm**

Fourier transforms decompose arbitrary functions into component sinusoids, which can highlight periodic patterns. For digital images, the sinusoidal elements of a 2D Fast
Fourier Transform (FFT) are plane waves (gratings), characterized by contrast, frequency, phase, and orientation. Operations applied to the frequency representation (reciprocal space) can be inverse-transformed to generate a filtered image. The hexagonal arrangement of typical ommatidia has 3 major axes (Figure 2 B), each approximated by a grating, and filtering frequencies higher than these generates a smooth image, with peaks near ommatidia centers that are easily detected with local maxima detection algorithms.

We developed a Python language module, the ommatidia detecting algorithm (ODA), which: (1) generates a 2D FFT, (2) finds the three fundamental gratings as the local maxima closest to the reciprocal image center, (3) filters higher image frequencies, (4) inverts the filtered 2D FFT, and (5) finds local maxima in the smoothed image (Figure 2 B). Users optionally check results in the reciprocal image with maxima superimposed.

The program stores ommatidia coordinates and calculates ommatidial diameter. An optional imported mask (a white silhouette on a black background) can help avoid false positives outside of the eye.

**Measuring Ommatidia using μCT**

We have further used the ODA to process 3D μCT data. This pipeline: (1) imports a stack of images representing a 3D dataset of points in the crystalline cones (Figure 3 A). Images can be edited to delete irrelevant pixels, and our program can pre-filter data by choosing a density range in a graphical interface, to further isolate crystalline cones, and can preview the whole dataset (Figure 4 A).

(2) projects coordinates onto 2D images and processes with the ODA (Figure 3 B). The layer of crystalline cones curves with little variation normal to its surface, allowing a sphere fit with least squares regression. The algorithm transforms points to spherical...
coordinates and interpolates a continuous surface, modeling the points’ radii as a function of elevation and azimuth. Finally, it partitions coordinates into shells of points inside and outside of the fitted surface (Figure 4 B).

(3) forms images of each shell by taking 2D histograms of elevation and azimuth (ignoring radius, Figure 3 C). Processing coordinates in 90°×90° segments, recentered before forming the histogram, avoids extreme spherical warping. The ODA approximates lens centers within each segment, then recombines them into the original coordinate system by finding the nearest groups of points between each shell. Finally, each point is labeled by its nearest center, effectively segmenting points into clusters of separate crystalline cones (Figure 4 C). Although Steps 2 and 3 work well for spherical eyes, when ommatidia are substantially skewed the offset between inside and outside shells prevents the algorithm from segmenting points into correct clusters, excluding them from the count. Therefore our program has an option for non-spherical ommatidia distributions where, instead of two shells in step 2, we extract one shell around the fitted surface containing 50% of residuals (Figure 4 B). Then as in 3, we partition the eye into 90° x 90° segments and find cluster centers with the ODA (Figure 4 C top and middle). We use the number of ommatidia from the algorithm to apply the spectral clustering algorithm to the total collection of points (Figure 4 C bottom), which was successful for a bumblebee but time consuming on larger moth scans.

(4) approximates the ommatidia count and lens diameters with clusters. If the initial ODA count in step 3 fails to match clusters of both shells for spherical eyes, or if clusters are not detected by the algorithm for non-spherical eyes, inspection of centers superimposed on the 2D histograms can determine the best count. The distance between adjacent
centroids approximates lens diameter. The ideal ommatidial axis is derived from planes formed by triplets of centers near the cluster, and we approximate the surface normal by averaging the normal vector for each plane. Singular value decomposition finds the longest semi-axis of the ellipsoid of a cluster to estimate the anatomical ommatidial axis. The angular difference between ideal and anatomical axes estimates anatomical skewness.

Ideally, anatomical axes of neighboring ommatidia would yield reliable IO angles, but results were highly variable. Especially in the bee scan, where angles were sometimes negative, the small cluster length compared to the intersection distance magnified the effect of angular deviations. While greater resolution, or using other structures to extend approximations closer to the intersection, could improve accuracy, we approximated anatomical IO angles by partitioning coordinates into evenly spaced vertical and horizontal sections (Figure 4 D), in which we projected clusters onto a parallel plane. For instance, for vertical sections, all clusters within a range of x values are considered and the 2D clusters formed by y and z values determine the vertical angle component. Nonlinear least squares regression fits a polynomial function of degree between 2 and 5, accepting higher degrees only when they reduce the sum of the squared residuals by at least 5%, and assuming IO angles are positive. This repeats independently for each vertical and horizontal section. The process approximates a horizontal and vertical subtended angle for each cluster pair and calculates the total angle as their hypotenuse. We call this hypotenuse the anatomical IO angle. While this method assumes one regression function per section, it allows different regression models per section and independent approximations for horizontal and vertical IO angles across the eye.
(Stavenga 1979). In addition, by keeping track of cluster pair orientation along the eye surface, we can calculate horizontal and vertical IO angles using the two-dimensional lattice proposed by Stavenga (1979; Figure 6C).

Finally, the program generates two spreadsheets: (1) for each crystalline cone cluster, the Cartesian and spherical coordinates of the centroid, the number and location of the points within its cluster, the approximate lens diameter, and the ideal and anatomical axis direction vectors are saved per row; and (2) for each pair of adjacent crystalline cones, the cones’ indexes from spreadsheet 1, the center coordinates for the two cones, the resulting orientation in polar coordinates, and the anatomical IO angle between the two are saved per row. These allow approximations of how spatial acuity, optical sensitivity, and the eye parameter (Snyder 1979) vary across the eye (Taylor et al. 2018). The full code for measuring ommatidia with µCT is available at GitHub where you can download the Python package and basic examples on how to use it (see Data Availability section).

**Results**

**Microscope Images**

We tested the ODA on images of flattened eye molds of six ants from 5 different species (Figure 5A). Automated counts were on average 97% (95.6 – 98.7%) of those taken by hand and they shared a strong and significant correlation (Pearson’s r=.99, df=4, p≪.001 two-tailed). The automated lens diameters were on average 99% (92.1 – 106.7%) of those taken by hand and also shared a strong and significant correlation (r=.82, df=4, p=.02).

We also tested microscope images of the eyes of 39 fruit flies (*D. melanogaster*; Figure 5B and C), to determine performance on direct images and comparisons within a species. Automated counts shared a strong and significant correlation with manual counts (r = .93,
df = 37, p ≪ .001). Automated counts were 845 ± 87 (mean±standard deviation) while hand counts were 810 ± 76, with automated counts equalling 104 ± 4% of those taken by hand. Though the difference in counts was not significant (t = 1.8, df = 37, p = 0.07), automated counts may exceed the hand counts because of partial ommatidia that were excluded during the manual counting process but not the algorithm. Automated diameters also shared a strong and significant correlation with manual diameters (r = .82, df = 37, p ≪ .001). Automated diameters were 16.3 ± 1µm while manual diameters were 15.6 ± .8µm, with automated diameters equaling 105 ± 3% of those taken by hand and is close to the 16µm taken previously (Franceschini 1972). Automatic diameters were greater (t = 4, df = 37, p < .001) possibly because manual calculations were averaged over several ommatidia in a row, which assumed straight, regular alignment. Facets actually curve slightly with the curvature of the eye, and can be interrupted by lattice abnormalities (Ready, Hanson, and Benzer 1976). However, lattice irregularities do not affect automatic diameter measurements as they are individual, and should equal at least those taken by averaging over a straight distance.

µCT

We tested ODA-3D on eye scans of two moth species (M. sexta and D. elpenor) that we collected, and one bumblebee (B. terrestris) used in Taylor et al. (2018; Figure 6). We additionally tested methods for non-spherical eyes on the bumblebee scan (Figure 7), including for features of an oval eye, regional changes in aperture and anatomical IO angle, and projecting onto world-referenced coordinates. The 2D histograms and the superimposed ommatidial centers suggested that the first count was more accurate for M. sexta while the second count was more accurate for D.
elpenor. This was because of substantial noise around the eye boundary in D. elpenor that we failed to remove in the prefilter stage. For B. terrestris, though the first count was more accurate than the second, it still missed many ommatidia visible in the dorsal region of the bumblebee eye maps of Figures 4 and 6. This was mostly due to differential exposure across the eye, resulting in many crystalline cones that were excluded at the pre-filter stage. As a result, our ommatidia counts were comparable to previous counts in the literature for the moths but not the bumblebee. The first and second counts were 103 – 108% and 93 – 98% of previous measurements for M. sexta (Stöckl, O’Carroll, and Warrant 2017; White 2003), 120% and 106% for D. elpenor (Stöckl et al. 2017), and, for B. terrestris, 85% and 64% of both the density-based count of the same scan (Taylor et al. 2018) and previous manual counts for conspecific worker bees (Streinzer and Spaethe 2014). These ommatidia had diameters that were highly consistent with measurements in the literature: 96 – 108% for M. sexta (Stöckl et al. 2017; Theobald, Warrant, and O’Carroll 2010; White 2003), 99 – 103% for D. elpenor (Stöckl et al. 2017; Theobald et al. 2010), and despite missing a large subsample of ommatidia, 102 – 104% for B. terrestris (Streinzer and Spaethe 2014; Taylor et al. 2018).

Both moth eyes showed ommatidial axes with minor skew from spherical alignment, potentially due to error estimating axes, or eye deformations during µCT preparations. The bee, however, showed axes with substantial skew, consistent with previous anatomical measurements (Baumgärtner 1928). Skew geometrically reduces effective lens diameter by the cosine of skew angle, and further still by refraction of the image space (Stavenga 1979). The adjustment without accounting for refraction produced marginal reductions for the spherical eyes: 0.5% in M.sexta and 0.7% in D. elpenor, but
more substantial reduction for the oval eye: 12.7% in *B. terrestris*. The spherical approximations for IO angle were close to anatomical IO angle in moth eyes but not the bumblebee eye: 106% in *M. sexta*, 85% in *D. elpenor*, and 32% in *B. terrestris*. Likewise, anatomical IO angles were consistent with previous measurements in the literature for moths but less for the bumblebee: 92 – 97% for the spherical and 98 – 103% for the anatomical approximation in *M. sexta*, (Stöckl et al. 2017; Theobald et al. 2010), 97 – 113% for the spherical and 114 – 133% for the anatomical approximation in *D. elpenor* (Stöckl et al. 2017; Theobald et al. 2010), but 39 – 46% for the spherical and 121 – 143% for the anatomical approximation in *B. terrestris* (Spaethe 2003; Taylor et al. 2018).

In bees, spherical coordinates largely account for vertical axis curvature, but vastly underestimated horizontal curvature. To better characterize their visual field, we projected the ommatidial axes onto a sphere outside of the eye, much like the world-referenced projection of Taylor et al. (2018; Figure 7C). However, instead of using the center of the head as our center, we used the center found in step B of ODA-3D, which is near but not exactly the center of the head. We chose a radius of 10 cm based on visual fixation behavior (Wehner and Flatt 1977). This world-referenced visual field has a FOV of about 140° horizontally and 140° vertically, agreeing exactly with previous measurements on the same scan that did not account for skewness (Taylor et al. 2018).

**Oval Eye Properties**

The bee eye is a non-spherical, oval eye, in which ommatidial axes intersect at different points for horizontal and vertical IO pairs (I_h and I_v in Figure 6C). Anatomical IO angles therefore should be separated into independent horizontal and vertical components (\(\Delta\phi_h\) and \(\Delta\phi_v\) in Figure 6C). In an oval eye: (1) the horizontal angle for horizontal IO pairs (0°
orientation) is $2\Delta\phi_h$ while the vertical angle is 0; (2) the horizontal and vertical angles for diagonal IO pairs ($\pm 60^\circ$ orientations) are $\Delta\phi_h^2$ and $\Delta\phi_v$; and (3) the proportion $\Delta\phi_v / \Delta\phi_h$ is $1/\sqrt{3}$ (Stavenga 1979). Our bee IO pair orientations followed a trimodal distribution with modes at $-59^\circ$, $0^\circ$, and $61^\circ$ (K Means algorithm with 3 means; $N = 11,626$, mean distance = $12^\circ$). We selected IO pairs within $15^\circ$ of centroids to accurately measure horizontal and vertical angles (Figure 7A). The bee fits several oval eye predictions: (1) horizontal angle for horizontal IO pairs, $2.94^\circ$, is nearly twice horizontal angle for diagonal pairs, $1.90^\circ + 1.36^\circ = 3.26^\circ$ while vertical angle, $0.08^\circ$, is close to $0^\circ$; and (2) the vertical angles for diagonal IO pairs, $.97^\circ$ and $.98^\circ$, are nearly equal. However, the horizontal angles for diagonal IO pairs, $1.90^\circ$ and $1.36^\circ$, which should be equal, differ by about $28\%$.

By combining the horizontal angle of diagonal pairs and half the horizontal angle of horizontal pairs, we approximated the horizontal anatomical IO angle component, $\Delta\phi_h = 1.48 \pm .88^\circ$ ($N = 7,503$). This is close to the range of $1.8^\circ – 3.3^\circ$ measured previously (Spaethe 2003). By combining the vertical angles of diagonal pairs we approximate $\Delta\phi_v = .95 \pm .60^\circ$ ($N = 4,828$), which is within the range of $0.6^\circ – 1.4^\circ$ measured in Spaethe (2003). Using these two anatomical IO angle components, the total anatomical IO angle $\Delta\phi = 1.76^\circ$, is closer to the previous measurements of $1.6^\circ$ and $1.9^\circ$ mentioned above. Also, the proportion $\Delta\phi_v / \Delta\phi_h = .64$ is close to $1/\sqrt{3} = 0.58$, as predicted for an oval eye. Interestingly, the anatomical IO angle components varied with elevation and azimuth (Figure 7B and C), which we binned into $15^\circ$ intervals. $\Delta\phi_v$ decreases by $.3^\circ$ horizontally from $-37.5^\circ$ azimuth to $7.5^\circ$ azimuth ($r = -.14$, df = 2174, $p \ll 0.001$) and then remains relatively constant up to $37.5^\circ$ azimuth ($r = 0.01$, df = 1,414, $p = 0.60$; Figure B, top left).
To our knowledge, this has not been measured previously but is consistent with the observation that the greatest overall acuity is in the central visual field (Baumgärtner 1928).

Vertically, $\Delta\phi_v$ decreases by $1.3^\circ$ from $-67.5^\circ$ to $22.5^\circ$ elevation ($r = -0.53$, df = 4870, $p \ll 0.001$) and then increases by $0.7^\circ$ up to $52.5^\circ$ elevation ($r = 0.29$, df = 2207, $p \ll 0.001$; Figure B, top right). This agrees with previous measurements showing a decrease from about $2.4^\circ$ ventrally to about $1^\circ$ centrally followed by a gradual increase to about $2^\circ$ dorsally. However, the previous measurement found a dramatic increase to $4^\circ$ at the dorsal extreme (Baumgärtner 1928), which we did not measure. This is likely due to technical differences: their bee species is unspecified, their specimen may be smaller and thus have a larger $\Delta\phi_v$ minimum than ours, and our eye scan lacks a substantial dorsal portion where they measured most of the increase (Baumgärtner 1928).

Horizontally, $\Delta\phi_h$ decreases by $0.8^\circ$ from $-67.5^\circ$ to $-22.5^\circ$ azimuth ($r = -0.03$, df = 6295, $p < 0.01$). This differs from the previous measurements taken on the same scan, which found that the anatomical IO angle increases from about $1.26^\circ$ to $1.90^\circ$ from the lateral to central eye with a strong positive correlation ($r = 0.50$, df = 3,471, $p \ll 0.001$; Taylor et al., 2018). This is likely because the previous measurements did not account for skewness, which we find increases laterally, and partially explains why our anatomical IO angle measurement exceeds theirs by about $0.16^\circ$. Vertically, $\Delta\phi_h$ decreases by $0.6^\circ$ from $-52.5^\circ$ to $7.5^\circ$ elevation, with an insignificant correlation ($r = 0.03$, df = 4,750, $p = 0.06$; Figure 7B, bottom right).

Adjusted lens diameter also varied with azimuth and elevation. Horizontally, it increases by $4 \mu m$ from $-37.5^\circ$ to $37.5^\circ$ azimuth ($r = 0.28$, df = 3,542, $p \ll 0.001$; Figure 7B,
bottom left, blue). The correlation largely reduces for absolute lens diameter ($r = 0.09$, df $= 3,542$, $p \ll 0.001$; Figure 7B, bottom left, blue dashed line). Likewise, adjusted lens diameter increases vertically by 5 µm from $-67.5^\circ$ to $7.5^\circ$ azimuth ($r = 0.25$, df $= 1,845$, $p \ll 0.001$; Figure 7B, top right, blue) while absolute lens diameter remains constant ($r = -0.01$, df $= 3,542$, $p = 0.8$; Figure 7B, top right, blue dashed line).

Skewness always reduces sensitivity but can lead to FOV and IO angle tradeoffs. In this eye, adjusted lens diameter, and consequently sensitivity, increases from the lateral to central visual field while vertical spatial acuity is highest near the center. Similarly, adjusted lens diameter, and consequently sensitivity, increases from the ventral to dorsal visual field. This results in high sensitivity and vertical spatial acuity near the central visual field at the expense of FOV, and greater FOV laterally and ventrally at the expense of sensitivity and vertical spatial acuity.

During visual fixation, bees incline their dorsal-ventral axis to $70^\circ$ (Wehner and Flatt 1977), adjusting our elevation parameter by $-20^\circ$, which appears to direct the region of optimal sensitivity and acuity ($0^\circ$ azimuth, $20^\circ$ elevation) towards visual targets.

Binocular overlap also occurs in the dorsomedial region with high vertical spatial acuity (Taylor et al. 2018). Similar tradeoffs are found in many other apposition eyes, but usually coincide with visible changes in diameter and curvature. In bees, though, the changes are not reflected in absolute lens diameters or IO angles that do not account for skewness, and were revealed only by analyzing skewness, which highlights both the flexibility gained by skewing ommatidia and its deceptive nature.
Discussion

Our methods successfully automate the estimation of multiple visual parameters of compound eyes. We tested compound eye images with the ODA, which filters frequencies based on the hexagonal arrangement of most ommatidia, and applies a local maximum detector to identify their centers. The ODA calculated ommatidial count and lens diameter from different media (eye molds, microscope images, and µCT scans), taxa (ants, flies, moths, and a bee), sizes (hundreds to tens of thousands of ommatidia), and eye type (apposition, neural superposition, and optical superposition). In all cases, measurements provided by the program matched with manual measurements on the same data, previous measurements in the literature, or both. Ommatidial counts were accurate, with an average proportion across all tested species of 93 – 102% (mean of low means – mean of high means%) of previous or manual measurements. Ommatidial diameters were even more accurate, with an average proportion across all tested species of 100.2 – 103.4% of previous or manual measurements.

The ODA-3D, which integrated the ODA into a µCT pipeline, proved successful on scans of two spherical moth eyes (*D. elpenor* and *M. sexta*) and one oval bee eye (*B. terrestris*). In addition to counts and diameters, ODA-3D estimated anatomical IO angles, FOV, and skewness, from which we calculated adjusted lens diameters to approximate the optical effects of skew. Skewness angles were insignificant in moth eyes, which generally require approximate spheracity for proper optical superposition. However the oval bumblebee eye showed significant skewness angles, implying reduced optical sensitivity. Again, estimates were consistent with manual measurements on the same data, previous measurements in the literature, or both. High resolution 3D data additionally offered
world-referenced coordinates and regional measurements across the visual field. We found regional changes in skewness, vertical spatial acuity, and sensitivity as well as regional three-way tradeoffs between acuity, FOV, and sensitivity caused by skewness. Traditional methods, such as histological sections, make it challenging to measure IO angles oriented along a perpendicular axis, where it is more efficient to measure multiple IO angles parallel to the sectioning plane than individual IO angles perpendicularly along many sections. With a 3D dataset the two are equally difficult, and across the bee eye we were able to measure horizontal resolution increasing vertically and vertical resolution increasing horizontally from the peripheral to the central visual field. As far as we know, this has not been measured previously, though it is consistent with previous measurements showing the greatest resolution in the central visual field (Baumgärtner 1928).

The great eye size range between and among invertebrate species (Casares and McGregor 2020; Currea et al. 2018; Gaspar et al. 2019; Land 1997; Land and Nilsson 2012; Shingleton et al. 2007), makes compound eyes ideal for studying environmental reaction norms and allometry. Little allometry research deals with compound eyes, and instead favors organs easily measured in one or two dimensions (McDonald et al. 2018; Shingleton et al. 2007; Shingleton, Mirth, and Bates 2008). By automating the more tedious tasks of characterizing compound eyes, our programs should help with this challenge. For instance, ODA counts and diameters allow total cell count approximations and correspond to the independent effects of cellular proliferation and growth during eye development. Further, our program facilitates measuring allometry of visual performance, addressing the environmental reaction norms of the anatomical determinants of vision.
Further, progress in understanding fruit fly eye development (D. melanogaster; Callier and Nijhout 2013; Casares and McGregor 2020; Gaspar et al. 2019; Ready et al. 1976), makes compound eyes ideal for assessing principles of eye development across different taxa (Casares and McGregor 2020; Friedrich 2003; Harzsch and Hafner 2006). And because optics are the first limit to incoming visual information (Land 1997; Stavenga 1979), they inform electrophysiological and behavioral data to infer intermediate neural processing (Currea et al. 2018; Gonzalez-Bellido, Wardill, and Juusola 2011; Juusola et al. 2017; Land and Nilsson 2012; Theobald et al. 2010; Wardill et al. 2017; Warrant 1999).

Our programs have some known limitations. Images for the ODA require sufficient resolution to properly detect ommatidia. For regular images, pixel length must be at most half the smallest ommatidial diameter. For μCT, if individual crystalline cones cannot be resolved at each layer, they are likely indiscriminable to the ODA. Further, some species, preparations, and scanning procedures, capture better contrast between crystalline cones and other structures while avoiding structural damage to the specimen. For example, M. sexta crystalline cones contrasted sharply with the background scan when prefiltered with just the threshold function. D. elpenor, however, had additional noise outside of the eye, and B. terrestris had uneven exposure, altering density measurements across locations in the scan and ultimately forcing omission of data. This may be an unintentional consequence of the contrast enhancing property of a synchrotron light source (Baird and Taylor 2017).

Ultimately, anatomical measurements cannot replace optical techniques in measuring compound eye optics (Stavenga 1979; Taylor et al. 2018). Light passing through an eye
refracts depending on the incident angle and index of refraction (Stavenga 1979). But our approximations used only incident angle, so our measurements of the aperture-diminishing effect of skewness represent lower bounds, and our measurements of IO angles are anatomical, not functional IO angles. Though skewness can be somewhat corrected for, nothing can match optical techniques (Stavenga 1979; Taylor et al. 2018).

Future work will be needed to understand the limitations of both the ODA and ODA-3D. Because the ODA depends on spatial frequencies corresponding inversely to the ommatidial diameter, an eye with a wide range of diameters may not work, and the ODA should be tested on eyes containing acute or sensitive zones, such as the robberfly (Wardill et al. 2017) and lattices with transitioning arrangements, such as hexagon to square in houseflies (Stavenga 1975) and male blowflies (Smith et al. 2015). Likewise, the ODA-3D should be tested on non-spherical non-oval eyes. While our program appropriately measured anatomical IO angles across the bumblebee’s oval eye and actually corroborated its oval eye properties, it may not work when IO angles change dramatically like in the robberfly (Wardill et al. 2017). Finally, the ODA-3D should be tested on non-insect arthropods.

Conclusion

Compound eyes are the most common eye type on Earth, found in nearly every ecological habitat and visual environment, and varying widely in size, shape, and architecture. Because they are diverse, ubiquitous, and subject to heavy selection pressure, they are crucial to understanding the evolution of vision. Our programs contribute to this effort and are open source, easy to install, easy to incorporate into
custom pipelines, and downloadable as Python modules. By successfully measuring parameters from a wide range of eye shapes and sizes, they should facilitate the study of the development, evolution, and ecology of visual systems in non-model organisms.

**Author Contributions**

JC: Conceptualization, Methodology, Software, Visualization, Writing- Original Draft.

YS: Conceptualization, Methodology, Writing- Review and Editing, Data Collection and Curation.

JT: Conceptualization, Methodology, Writing- Review and Editing, Supervision, Funding acquisition.

AK: Conceptualization, Methodology, Writing- Review and Editing, Funding acquisition.

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number NE/P003915/1 to IJK. Gavin Taylor and Emily Baird shared their CT scans of *B. terrestris*. Michael Reisser and Arthur Zhao provided valuable discussions regarding the µCT pipeline.

**Code Availability**


**Data Availability**

All datasets are freely available at https://doi.org/10.6084/m9.figshare.c.5303165.v1 and are itemized in the table below. The image stack of the µCT data for the *B. terrestris* scan were drawn from Taylor et al. (2018) and are available at https://www.morphosource.org/Detail/ProjectDetail/Show/project_id/646.

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Figures

Figure 1: Diagrams of apposition and superposition eyes demonstrating the geometric tradeoffs between lens diameter (D), interommatidial angle (Δφ), and field of view (FOV) for spherical (A. and B.) and non-spherical eyes (C. and D.).

A. In spherical apposition eyes, D directly determines sensitivity while Δφ inversely determines acuity. B. In superposition eyes, migrating pigment (indicated by the arrows) allows the ommatidia to share light, increasing the eye’s sensitivity. As a result, these eyes must adhere to a spherical design.

C-D. In non-spherical eyes, the intersection of ommatidial axes differs from the center of curvature, with ommatidial axes askew from the surface of the eye. Consequently, FOV and Δφ are not externally measurable and the effect of D on sensitivity is reduced by greater angles of skewness. C. When the distance to the intersection is greater than the radius of curvature, FOV and Δφ decrease, increasing average spatial acuity by directing more ommatidia over a smaller total angle. D. Inversely, when the distance to the intersection is less than the radius of curvature, FOV and Δφ increase, decreasing average spatial acuity by directing fewer ommatidia over a smaller total angle. In both cases, optical sensitivity is lost because skewness reduces the effective aperture of the ommatidia.
Figure 2: The ommatidia detecting algorithm (ODA) extracts periodic signals in a 2D image using the FFT. A. In the frequency domain of a 2D FFT, called the reciprocal space, gratings are represented by an x- and y-frequency. The polar coordinates represent visual properties of the corresponding grating. The radial distance is a grating's spatial frequency, with high frequencies farther from the origin. The polar angle is the grating's orientation, which is perpendicular to the grating’s wavefront. Notice that the reciprocal space has local maxima (in red) approximately equal to the input grating parameters (polar angle=45° and radial distance=.047±.005). B. In a hexagonal lattice, there are three major axes (here in blue, green, and red). Each axis corresponds to a 2D spatial frequency (and it’s negative), visible in the image’s reciprocal space. The periodic nature of the axes results in harmonic frequencies. A low-pass filter returns a version of our original image primarily representing these three axes. The center of each ommatidium is found at the local maxima of the filtered image.
A. Import Stack

B. Get Cross-Sections
outside
inside

C. Find Ommatidial Clusters

D. Measure Visual Parameters

FOV

D1, D2
Figure 3: ODA-3D starts with an image stack, which may be pre-filtered and cleaned of unrelated structures. Then we filter the relevant density values and fit a surface to the coordinates, allowing us to split the points into two sets or cross sections, inside and outside the fitted surface. For each cross section, we generate spherically projected, rasterized images that are processed by the FFT method for locating ommatidia, yielding approximate centers for the ommatidia. With these centers, we can find the coordinate clusters corresponding to independent ommatidia and automatically measure eye parameters.
Figure 4: Checkpoints along ODA-3D for the bumblebee scan. **A. Import Stack:** The bumblebee ODA-3D pipeline started with an image stack that we pre-filtered and manually cleaned of unrelated structures. **B. Get Cross-Sections:** A surface was fitted to the coordinates identifying a cross-section of points within 50% of the residuals. We generated a spherically projected, 2D histogram of the crystalline cone coordinates (in grayscale). **C. Find Ommatidial Clusters:** The inset zooms into stages of applying the ODA to label individual crystalline cones. The ODA locates the cluster centers (red dots in top), allowing us to partition the coordinates based on their distance to those centers (boundaries indicated by the red lines in middle). Then we apply the spectral clustering algorithm to all of the 3D coordinates using the ommatidia count (bottom), finding the clusters approximating the individual crystalline cones (each colored and labelled separately in bottom). **D. Measure Visual Parameters:** Ommatidial diameter corresponds to the average distance between clusters and their adjacent neighbors. Ommatidial count corresponds to the number of clusters. For non-spherical eyes, we can
partition the 3D coordinates into vertical (*left*) and horizontal (*right*) cross-sections in order to calculate independent vertical and horizontal anatomical IO angle components accounting for skewness in the ommatidial axes. The insets zoom into regions around the 25th, 50th, and 75th percentile ommatidia along the y-axis (*left*) and x-axis (*right*). Note: in C and D the different colors signify different crystalline cone clusters. In D, the black lines follow the ommatidial axes and the black dots indicate the cluster centroids.
Figure 5: The ODA successfully approximated ommatidial counts and diameters when compared to manual measurements. A. When applied to 6 ant eye molds of 5 species ranging in overall size and lattice regularity, the automated counts were 97% and the diameters were 99% of those measured by hand. For each image, the program missed relatively few ommatidia and the lens diameter measurements were successful even when they varied substantially within an image (as in #5 from the left). Species from left to right: Notoncus ecatommoides, N. ecatommoides, Rhytidoponera inornata, Camponotus aeneopilosus, Myrmecia nigrocincta, and Myrmecia tarsata. B. When applied to 39 microscope images of fruit fly eyes from the same species (D. melanogaster), the automated counts were 104% and the diameters were 105% of those measured by hand, with correlations of .93 and .82. C. Two example fruit fly eyes, one small and one large, with the automated ommatidia centers superimposed as white dots. Again, there were relatively low rates of false positives and negatives. Scale bars are 50µm.
Figure 6: Comparing visual fields based on the spherical approximation. A. ODA-3D allows us to map the visual fields of our three specimens. Using their spherical projection, we map the azimuth (along the lateral-medial axis) and elevation (along the ventral-dorsal axis) angles of ommatidia and represent lens diameter with color. The colorbar to the right indicates lens diameter, and shows diameter histograms for each species in white. Insets zoom in on a 20°x20° region in the center of each eye, showing ommatidial lattices in more detail. Note that the spherical projection of the bee eye underestimates its visual field and is missing a portion dorsally, as explained in the text. B. Optical parameters for the three species are presented as a table. Values are mean ± s.d except for angular measurements, which show median (IQR). Comparable measurements from the literature are in grey below corresponding values. References are signified in superscript letters: a White (2003), b Stöckl et al. (2017), c Theobald et al. (2010), d Taylor et al. (2018), e Streinzer and Spaethe (2014), f Baumgartner (1928), g Spaethe (2003). C. Adapted from Stavenga, 1979. Bee eyes are approximately oval, and, unlike spherical eyes, have different intersection points for horizontal (Ih) and vertical (Iv) ommatidial pairs corresponding to different IO angle components horizontally (Δφh) and vertically (Δφv). For oval eyes, the horizontal component of the IO angle of horizontal pairs (orientation=0°) is 2Δφh while the vertical component is ~0° and the horizontal component of diagonal pairs (orientation=±60°) is Δφh while the vertical component is Δφv. Because the ommatidia form an approximately regular hexagonal lattice, Δφv/Δφh = 1/√3 for spherical eyes, and √3 for oval eyes.
Figure 7: Mapping optical parameters onto the visual field of B. terrestris. 

A. Grayscale heatmaps indicate kernel density estimation of the entire dataset and red lines indicate statistics about surrounding clusters: the longer, half opacity lines indicate IQR, tick marks indicate the 99% CI of the median using bootstrapping, and points (often overlapping with the tick marks) indicate the median. Solid blue lines, ticks, and points indicate the median and IQR of adjusted lens diameters in the same way as red lines, and correspond to inserted axes on the right. Dashed blue lines indicate the median absolute lens diameters also corresponding to the axes on the right. 

A. The vertical and horizontal subtended angles of ommatidial pairs with respect to their orientation fit well into the oval eye lattice from Figure 6C. The pair orientations form a trimodal distribution with means close to ±60° (diagonal pairs) and 0° (horizontal pairs). Medians, IQRs, and 99% C.I.s are plotted for each orientation ±15°. 

B. Using the lattice described in Figure 6C, we convert the horizontal and vertical subtended angles into the horizontal and vertical IO angle components (Δφ_h and Δφ_v) and plot them with respect to their azimuth and elevation positions on the projected visual field. Skewness varies systematically across the eye influencing adjusted lens diameters and the horizontal and vertical IO angle components (Δφ_h and Δφ_v). The position of each IO angle component is the average position of the two ommatidia per IO pair.
CHAPTER III
SMALL FRUIT FLIES SACRIFICE TEMPORAL ACUITY TO MAINTAIN
CONTRAST SENSITIVITY

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Abstract

For holometabolous insects like the fruit fly, growth is restricted to the larval stages of development and limited larval feeding, common in nature, results in smaller adult flies. Despite the importance of vision to flies, smaller adults possess smaller eyes that, in principle, must sacrifice spatial acuity or contrast sensitivity due to smaller optics. Because fruit fly vision is currently understood from uniformly large, lab-reared adults, how early development affects adult vision is unknown. Do smaller eyes sacrifice spatial acuity, by increasing their inter-ommatidial angles, or sensitivity, by decreasing their ommatidial diameters? Further, might the visual system neurally adapt to these optical constraints via temporal or spatial pooling? To address these questions, we first generate a broad distribution of body (1.67-2.34mm; n=24) and eye lengths (0.33-0.44mm; n=24) by removing larvae from their food during their third instar, resulting in flies more similar to those in the wild. Then, we measure the optical sacrifices of small eyes, finding that smaller eyes (0.19 vs. 0.07mm²) have substantially fewer (978 vs. 540, n=45) and smaller ommatidia (222 vs. 121μm²; n=45) separated by slightly wider inter-ommatidial angles (4.5 vs. 5.5°; n=34). This corresponds to a greater loss in contrast sensitivity (≤50%) than spatial acuity (≤20%). Finally, using a flight arena and psychophysics paradigm, we find that smaller flies lose little spatial acuity (0.126 vs. 0.118CPD; n=45), and recover contrast sensitivity (2.22 for both; n=65) by sacrificing temporal acuity (26.3 vs. 10.8Hz; n=112) at the neural level. Therefore smaller flies sacrifice contrast sensitivity to maintain spatial acuity at the optical level, but recover contrast sensitivity, almost completely, by sacrificing temporal acuity at the neural level.

Keywords: acuity, sensitivity, development, plasticity, drosophila, fruit fly
1. Introduction

In general, larger animals have eyes that are larger in absolute terms but smaller relative to body size (Hughes, 1977; Rensch, 1948; Stevenson et al., 1995). Because optical quality is limited by the eyes’ absolute and not relative size (Land and Nilsson, 2012), progressively smaller animals face an increasingly difficult optical challenge. Substantial comparative work has demonstrated evolutionary adaptations in the optics and neural processing of visual systems to cope with small apertures (Hughes, 1977; Krapp, 2000; Land and Nilsson, 2012; Theobald et al., 2010). However, though body and eye size can also vary substantially within species (Shingleton et al., 2007; Shingleton et al., 2009), little is known about what developmental adaptations smaller-eyed conspecifics employ. The fruit fly, with two neural superposition compound eyes, each about 0.15 mm$^2$ in area, exemplifies this small-eyed developmental challenge. Limited food availability during the fruit fly’s late larval stages, a common condition in nature, results in smaller adults with smaller eyes (Callier and Nijhout, 2013; Shingleton et al., 2007; Shingleton et al., 2009). Each eye is an approximate hemisphere composed of about 800 nearly identical ommatidia, each containing 1 lens that focuses light upon 8 photoreceptors (Ready et al., 1976). This geometric arrangement dictates that smaller eyes must confer poorer vision due to a decrease in the size of each ommatidial lens, an increase in the angle between ommatidia, or some combination of both. Neural summation processes might compensate for some of this loss, but only at the expense of some form of acuity (Warrant, 1999). Although small adults are common in nature where larval food availability and other environmental factors are highly variable, fly vision is mostly studied with uniformly
large, lab-reared adults, and how small adults cope with small optics is unknown. Here we measure the sacrifices made by smaller flies at the optical level, and the summation processes they employ at later stages.

1.1. Limited larval feeding leads to adult flies with small eyes.

The size of a holometabolous insect in general, and a fruit fly in particular, is determined by the size it achieves as a larva (Shingleton et al., 2008). Fruit fly larval development is divided into a sequence of 3 instars and allocates much of its nutrient intake towards growth. During the last instar, a larva eats until it reaches a critical size, eventually stops feeding, and wanders away from the food source in search of a place to pupate (Callier and Nijhout, 2013; Edgar, 2006; Shingleton et al., 2007). Importantly, there is a delay between when the larva reaches critical size and when it begins wandering, called the ‘interval to cessation of growth’ or the ‘terminal growth period’ (TGP; Callier and Nijhout, 2013; Edgar, 2006; Shingleton et al., 2007). During the TGP, larvae will continue to feed if possible but exposure to starvation or limited nutrition results in smaller but otherwise normal adults (Callier and Nijhout, 2013; Edgar, 2006). This developmental plasticity allows feeding that may be suboptimal for growth but necessary for survival (Edgar, 2006; Shingleton et al., 2008; Stevenson et al., 1995).

The effect of larval feeding on the developing eye is similar to and affected by the development of the overall body. Each kind of imaginal disc (eye-antenna, leg, and so on) has its own critical size and TGP. Limited nutrition during the TGP of an imaginal disc results in slower rates of growth and proliferation and, eventually, a smaller adult organ. (Shingleton et al., 2007) In the case of the fruit fly’s eye imaginal disc, limited nutrition
during the third instar results in small adult eyes (Callier and Nijhout, 2013; Edgar, 2006; Shingleton et al., 2009; see Figure 1 A and B).

1.2. Smaller eyes must sacrifice spatial acuity, contrast sensitivity, or some combination of both.

A general principle of vision is that spatial acuity, or visual sharpness, and contrast sensitivity, or the ability to discriminate luminance levels, trade off (Land, 1997; Land and Nilsson, 2012; Theobald et al., 2010). Contrast sensitivity is determined by the amount of light absorbed by each photoreceptor, which is limited in the fruit fly by ommatidial diameter (Figure 1 C, labeled D). The contrast sensitivity, \( S \), of an eye to an extended light source is calculated by: \[
S = \frac{D}{d \cdot l \cdot k}
\]
where \( D \) is ommatidial diameter (\( \mu m \)), \( f \) is ommatidial focal length (\( \mu m \)), and \( d, l, \) and \( k \) the diameter(\( \mu m \)), length (\( \mu m \)), and absorption coefficient (photons \( \mu m^{-1} \)) of each photoreceptor rhabdomere (Warrant and Nilsson, 1998). Spatial acuity is inversely determined by the angle between adjacent ommatidia, the inter-ommatidial angle (Figure 1 C, labeled \( \Delta \phi \); Land and Nilsson, 2012). The highest discernible spatial frequency, \( v_{max} \), of a hexagonal lattice like the fruit fly’s eye is given by: \( \frac{1}{2 \cdot \Delta \phi} \). The fundamental acuity-sensitivity tradeoff is demonstrated by the eye’s geometry, such that decreasing \( \Delta \phi \), which increases spatial acuity, necessarily decreases \( D \), which decreases contrast sensitivity, and vice-versa (Land and Nilsson, 2012).

Likewise, reducing eye size necessarily decreases \( D \), increases \( \Delta \phi \), or some combination of both. As a result, smaller flies, who have smaller eyes, must sacrifice at least one of the two visual properties, acuity or sensitivity, and the overall image quality.

Because the development of the imaginal discs is largely influenced by feeding, and this effect can vary between the different imaginal discs (Shingleton et al., 2009), it is
unknown how limited larval feeding will affect the optics of small eyes. For most imaginal discs, nutrition limits both cell proliferation and cell growth, resulting in adult organs that are smaller due to both fewer and smaller cells (Robertson, 1963; Shingleton et al., 2009). If this holds for the eye imaginal disc, then smaller flies could have fewer and smaller ommatidia, necessarily reducing contrast sensitivity and possibly reducing spatial acuity.

1.3. Neural summation can improve contrast sensitivity, but only at the expense of spatial or temporal acuity.

Low light absorption due to smaller ommatidia presents the same problem as that faced by all animals viewing images in dim light: how to resolve an accurate image with fewer photons? Both vertebrate and invertebrate visual systems improve the visible range of ambient light intensities by increasing the receptive field of visual interneurons, via spatial summation, or increasing the integration time of phototransduction, via temporal summation (Warrant, 1999; Warrant and Nilsson, 2006). However, spatial and temporal summation strategies improve contrast sensitivity only by sacrificing spatial and temporal acuity. Spatial summation increases the functional inter-receptor angle, improving contrast sensitivity while sacrificing spatial acuity according to the acuity-sensitivity tradeoff discussed in Section 1.2. The fruit fly has demonstrated spatial summation in response to optic flow, quickly reducing their peripheral spatial acuity from about .1CPD to .07CPD to improve contrast sensitivity in regions most affected by motion blur (Theobald, 2017). Similarly, temporal summation allows for greater photon capture but reduces temporal acuity, or the fastest discernible change in luminance. During dark adaptation, fruit fly photoreceptors increase integration time and consequently restrict
themselves to a lower bandwidth of temporal frequencies, reducing their temporal acuity (Juusola and Hardie, 2001). Intracellular measurements show that fruit fly temporal acuity decreases approximately from 30Hz to 10Hz over a 4 log unit reduction in ambient light (Juusola and Hardie, 2001; depending on methodology, a higher values have been measured for temporal acuity; for example, see Cosens and Spatz, 1978). Reductions in temporal acuity can be detrimental for quickly moving animals and animals' use of spatial versus temporal summation corresponds greatly to their visual ecology (Krapp, 2000; Theobald et al., 2010; Warrant, 1999).

1.4. Fly vision is mostly understood from uniformly large, lab-reared adults.

Conventions of lab husbandry rear fruit flies that are a skewed representation of natural fruit fly populations. The abundance of food and lack of competition and predation in lab environments enable larvae to grow to an ideal size with large eyes. This is ideal to minimize experimental variation, but because smaller eyes necessarily confer poorer vision (Land, 1997; Land and Nilsson, 2012), it is unknown how smaller flies developmentally adapt to this optical challenge. To examine these effects, we leverage the known effect of larval feeding on adult eye sizes to generate variable adult sizes like those found in nature (Figure 1 A), by removing larvae from their food source during their third instar but prior to the wandering stage. Then, we measure the scaling relations of ommatidial count, ommatidial area, and inter-ommatidial angle in relation to eye area to approximate the spatial acuity and contrast sensitivity sacrifices that small eyes make at the optical level. Finally, we measure to what extent small fruit flies use spatial or temporal summation, as demonstrated in their behavior, to recover some of the contrast sensitivity lost by their small optics. We demonstrate that smaller eyes, due to restricted
larval diet, maintain roughly the same inter-ommatidial angle as their larger peers by
developing both fewer and smaller ommatidia, sacrificing contrast sensitivity more than
spatial acuity at the optic level. However, we further demonstrate that smaller flies
maintain the same contrast sensitivity as their larger peers by sacrificing temporal acuity
at the neural level. Therefore, small fruit flies have smaller eyes and slower, but
otherwise normal vision.

2. Materials and Methods

2.1. Subjects

For body-eye size comparisons (Section 3.1), we compared 4 conditions: lab reared flies
exposed to abundant larval feeding ('lab'; \( n = 39 \)), lab-reared flies exposed to restricted
larval feeding ('lab-restricted'; \( n = 24 \)), recently caught wild flies ('wild'; \( n = 45 \)), and the
progeny of wild caught flies exposed to abundant larval feeding ('wild-fed'; \( n = 40 \)). For
eye allometry and behavior experiments (Sections 3.2 – 3.4), only lab-restricted larvae
were used. All individuals were identified as \textit{Drosophila melanogaster} (Meigen) upon
visual inspection, and wild caught flies were additionally bred with lab flies to ensure two
generations of viable offspring were produced.

All lab, lab-restricted, and wild-fed larvae were fed standard media and reared at 21°C on
a 12 h:12 h light: dark cycle. Lab and wild-fed larvae continued into adulthood in the
same manner. For lab-restricted flies, during their third instar and prior to the wandering
stage, larvae were separated from the media using a sieve and running water and were
placed in a jar with moisture but without media. Upon eclosion, adults were transferred to
a jar containing standard media in abundance. 3-6 days after eclosion, adults were
cold-anesthetized and glued to a rigid tungsten rod, 0.02 mm in diameter, on the dorsal
prothorax. After recovering for about an hour, situated upside-down with a piece of paper on their feet to prevent them from beating their wings, they were suspended at the center of the immersive flight arena for psychophysics experiments (Cabrera and Theobald, 2013). After testing, the flies’ head was glued to the thorax (to prevent movement during microscopy) for the measurement of optical parameters.

For measuring optical parameters, \( n = 45 \) flies were used to measure eye area, ommatidial count, and ommatidial area and a different set of \( n = 34 \) flies were used to measure eye area, ommatidial area, and inter-ommatidial angle. For behavioral measures, \( n = 65 \) flies were used to measure contrast sensitivity, \( n = 45 \) flies were used to measure spatial acuity, and \( n = 112 \) flies were used to measure temporal acuity with some overlap.

2.2. Optical Parameters

Body length, ommatidial count, eye area, and ommatidial area were measured using a digital recording microscope (Zeiss Axio Scope.A1) and a custom python script using human input. Body length was measured as the shortest distance from the tip of the head to the end of the abdomen. For eye measurements, multiple images (~20) were taken per fly from one angle, at fixed intervals of focus depth. A custom python script generated a single image composite of the stack of photos, using the Sobel operator to choose the pixel of highest focus from the stack of images. The final focus stack displays the entire eye in focus, allowing us to count the ommatidia and take direct measurements on a computer display. The projected eye area was calculated by fitting an ellipse to the contour of the eye. Ommatidial area was approximated by averaging the length of 81 ommatidia near the center of the eye and approximating the lens as a circle.
Inter-ommatidial angle was measured for each fly by using a precise goniometer under a
digital recording microscope. Using the pseudopupil as a guide, the eye was positioned so
that (1) the microscope stared directly down an ommatidium and (2) the center of rotation
of the eye matched that of the goniometer. The initial angle on the goniometer was
recorded and the eye was pitched by 20 one-degree intervals. The distance covered by 6
ommatidia was measured at each interval and then averaged. This approximates the 1°
arc of the eye, L. Notice that the proportion of the inter-ommatidial angle to one degree is
equal to the proportion of the ommatidial diameter, D, to the one degree arc, L, allowing
us to approximate the inter-ommatidial angle as, $\Delta \phi = D/L$, in degrees. (Land, 1997)

2.3. Allometry

Allometric scaling between physical traits usually follow a power law, $Y = aX^b$, where $Y$
and $X$ are traits and $a$ and $b$ are constants (Shingleton et al., 2007; Voje et al., 2014). $b$
is known as the allometric constant and represents the rate of growth of $Y$ in comparison to
$X$. $b = 1$ implies isometry or 1:1 scaling between $X$ and $Y$; $b < 1$ implies hypoallometry, so
that as $X$ increases, $Y$ increases at a decreasing rate; $b > 1$ implies hyperallometry, so that
as $X$ increases, $Y$ increases at an increasing rate. These same interpretations apply for $b < 0$
except they imply inverse allometries, so that as $X$ increases $Y$ may decrease
isometrically ($b = -1$), hypoallometrically ($b > -1$), or hyperallometrically ($b < -1$).
Approximations for $a$ and $b$ are calculated by log-transforming the allometric model,
$log(Y) = log(a) + b \cdot log(X)$, and using ordinary least squares regression.

2.4. Flight arena

Our cube arena uses four first-surface mirrors, angled at 45 deg to each side, to project a
stimulus on back-projection screen material inlaid on five sides (Figure 1, D-F). For
details on the flight arena, see Cabrera and Theobald, (2013). This study uses only the front face of the arena, which displays 229X229 pixels. The experiments were performed with room lights on, producing a Michelson contrast between dark and light areas in the arena of 85% (Caballero et al., 2015).

In the arena, the immersed fly tries to minimize retinal slip, or perception of motion, by steering as if it were untethered (Götz, 1987; Tammero et al., 2004). The dorsal tether immobilizes the fly but leaves the wing beats unaltered. Wing beats are captured by an infrared light emitting diode, invisible to the fly, that casts shadows of each wing onto a pair of photodiodes as the wing beats. The difference between the left and right wing beat amplitude (ΔWBA) is proportional to yaw torque (Figure 1, F; (Götz, 1987; Tammero et al., 2004)). The amplitude difference was visibly obvious in real-time when flies were exposed to involuntary optic flow.

2.5. Stimulus

Each experiment consists of open-loop sequences of moving sinusoidal gratings interspersed by 3 s bouts of closed-loop fixation of a striped bar. During fixation, the wing beats control the position of the rotating vertical bar, which improves their responsiveness to experimental presentations (Heisenberg and Wolf, 1979; Reichardt and Wenking, 1969). During the test sequences, grayscale sinusoidal moving gratings from a list of spatial frequencies, temporal frequencies, and contrasts, moving either to the left or right, were presented in a randomized order. Each grating was presented for 1 s, followed by the fixation task, until each fly was exposed to the whole list.
2.6. Moving Sinusoidal Gratings

The moving sinusoidal grating permits the independent manipulation of contrast, spatial frequencies, and temporal frequencies (a single frame can be seen in Figure 1, on the front of the arena in D and E). The two-dimensional grating is represented at each spatial coordinate, \((x,y)\), and time, \(t\), by a sine function: \(c(x,y,t)\), where \(c\) is contrast, representing the ratio between the lightest and darkest parts of the grating; \(f_s\) is spatial frequency, representing the frequency of luminance change over distance; and \(f_t\) is temporal frequency, representing the rate of change of the grating’s phase over time. Contrast is measured as the Michelson contrast of the projected grating: frequency refers to the contrast frequency of the sinusoidal pattern moving at constant speed and not, for instance, the refresh rate of the projector or the oscillation frequency of an oscillating bar. The sine gratings were oriented so that the contrast bands were vertical (see front panel of Figure 1) and the motion was to the left or right.

3. Results

3.1. Limited nutrition leads to smaller eyes, as it does in nature.

One-way analysis of variance tests demonstrate that at least one of the four conditions is significantly different in both body (\(F(144, 3)=28.60, p<0.01\)) and eye size (\(F(144, 3)=71.84, p<0.01\)). Post hoc analysis using Tukey's honestly significant difference test is used to assess pairwise differences. Compared to wild flies, lab flies have an approximately 13% longer body (95%CI=[0.16, 0.48]mm, \(p<0.01\)) and 15% larger eyes (95%CI=[0.05, 0.10]mm, \(p<0.01\)). Compared to lab flies, lab-restricted flies have an approximately 19% smaller body (95%CI=[0.26, 0.64]mm, \(p<0.01\)) and 24% smaller eyes (95%CI=[0.09, 0.15]mm, \(p<0.01\)). Lab-restricted flies are the same size as wild
flies (95% C.I. = [-0.31, 0.06] mm, ns) and have eyes that are about 6% smaller than wild flies (95% C.I. = [-0.07, -0.01], mm p<0.01). Finally, wild-fed flies are the same size (95% C.I. = [-0.21, 0.12] mm, ns) and have the same eye size (95% C.I. = [-0.04, 0.01] mm, ns) as lab flies.

Despite some differences in absolute sizes, the scaling relations of eye length and body length did not differ substantially between groups. Each scaling relation fits well into the allometric model (lab: p<0.01, R²=0.40; wild: p<0.01, R²=0.60; lab-restricted: p<0.01, R²=0.44; wild-fed: p<0.01, R²=0.56). For each condition, eye length scales hypoallometrically with respect to body length (lab: b=0.41, 95% C.I. = [0.23, 0.59]; wild: b=0.68, 95% C.I. = [0.51, 0.85]; lab-restricted: b=0.69, 95% C.I. = [0.35, 1.03]; wild-fed: b=0.47, 95% C.I. = [0.33, 0.61]). While the allometric constants do not differ greatly (their confidence intervals overlap), they are more similar between larval feeding conditions than related individuals: the constant for lab flies, b=0.41, is closer to that of wild-fed flies, b=0.47, than lab-restricted flies, b=0.69; the constant for wild flies, b=0.68, is closer to that of lab-restricted flies, b=0.69, than wild-fed flies, b=0.47. Larval feeding, therefore, is a strong predictor of adult eye to body scaling relations and restricting larval feeding of lab bred flies generates a range of adult body and eye sizes more similar to those in the wild.

3.2. Small eyes sacrifice contrast sensitivity more than spatial acuity at the optical level.

The scaling of both ommatidial count and ommatidial area with eye area (Figure 3 A and B) each fit well into the allometric model (p<0.001 for both and R²=0.951 and R²=0.954, respectively). Ommatidial count scales positively and hypoallometrically with respect to eye area (b=0.58, 95% C.I. = [0.538, 0.629]; a=2,502, 95% C.I. = [2,310, 2,710]), meaning
that smaller eyes have fewer ommatidia but have a higher ratio of ommatidial count to eye area, or ommatidial density. Ommatidial count ranged from 540 ommatidia in the smallest eye to 978 ommatidia in the largest, an increase of 45%. Inversely, ommatidial densities ranged from 4,970 ommatidia mm\(^{-2}\) in the largest eye to 7,833 ommatidia mm\(^{-2}\) in the smallest eye, an increase of 37%.

Ommatidial area also scales positively and hypoallometrically with respect to eye area \((b=0.57, 95\% \text{ C.I.}=[0.531, 0.608]; a=643.4, 95\% \text{ C.I.}=[596, 695])\), meaning that smaller eyes have smaller ommatidia in absolute terms, but larger ommatidia in proportion to the overall eye.

The scaling of inter-ommatidial angle with eye area (Figure 3 C) fits well into the allometric model \((p<0.001 \text{ and } R^2=0.31)\). Inter-ommatidial angle scales inversely and hypoallometrically with eye size \((b=-0.21, 95\% \text{ C.I.}=[-0.329, -0.099]; a=1.12, 95\% \text{ C.I.}=[0.894, 1.351])\), meaning that smaller eyes have disproportionately wider inter-ommatidial angles.

Using the equations for contrast sensitivity and spatial acuity in section 1.2, we can compare how the two visual properties scale with respect to eye size (Figure 3 D), due to their dependence on ommatidial area and inter-ommatidial angle. By normalizing the allometries so they both equal 1 for the largest eye, we can ignore the scaling factor, \(a\), and compare their respective rates of growth or allometric constants. Both fit well into the allometric model (sensitivity: \(p<0.001, R^2=0.953\); acuity: \(p<0.001, R^2=0.289\)) and scale positively and hypoallometrically with eye area, though contrast sensitivity scales at a higher rate than spatial acuity (sensitivity: \(b=0.57, 95\% \text{ C.I.}=[0.531, 0.608]; a=0.21, 95\% \text{ C.I.}=[0.099, 0.329])\). Therefore, smaller flies sacrifice contrast sensitivity to
a greater extent than spatial acuity. For instance, a large and small fly might have average inter-ommatidial angles of about 4.5° and 5.5°, respectively, resolving spatial frequencies up to 0.13 and 0.10 cycles per degree. This corresponds to a sacrifice of about 20% in spatial acuity for the smaller fly. Meanwhile, a large and small fly might have ommatidial areas of about 222μm² and 121μm², respectively. Because contrast sensitivity is directly related to ommatidial area, this corresponds to a sacrifice of about 45% in contrast sensitivity for the smaller fly.

3.3. Diffraction is minimally affected by eye sizes in this range.

The fruit fly’s already small ommatidia suggest that smaller eyes might be further affected by the diffraction of light. A diffraction limited eye would have a lens resolution equal to the spatial acuity of the eye: \( \frac{\lambda}{D} = 2 \cdot \Delta \phi \) (Howard and Snyder, 1983). However, eye resolutions are usually less than their lens resolution (Howard and Snyder, 1983; Wehner, 1981), such that the maximum wavelength of light that can be resolved, \( \lambda \), is given by: \( \lambda < 2D \cdot \Delta \phi \). Using this metric, we found that the maximum wavelength had no significant scaling relation with eye size (Figure 3 E; \( p=0.125, R^2=0.072; b=-0.1 \), \( 95\% \text{C.I.}=[-0.233,0.030] \); \( a=2208 \), \( 95\% \text{C.I.}=[1703,2870] \)), suggesting that ommatidial diameter and inter-ommatidial angle trade off appropriately to minimize the diffraction effects of smaller optics.

3.4. Behavior

To measure the functional implications of small eyes, we conducted three psychophysics experiments using moving sinusoidal gratings to measure (1) contrast sensitivity, (2) spatial acuity, and (3) temporal acuity (Figure 4). Contrast sensitivity was measured by displaying 32 gratings with 16 different contrasts ranging from 0 (completely gray) to
0.85 (the maximum we could generate with lights on) at equal intervals, moving left or right, with a spatial frequency of 0.05 cycles per degree (CPD) and temporal frequency of 10Hz. Spatial acuity was measured by displaying 64 gratings at 32 different spatial frequencies ranging from 0.05 to 0.16 CPD at equal intervals, moving left or right, with a contrast of 0.85 and temporal frequency of 10 Hz. Finally, to measure temporal acuity, 64 gratings were presented from 32 different temporal frequencies ranging from 1 to 100 Hz at logarithmically spaced intervals, moving left or right, with a contrast of 0.85 and spatial frequency of 0.05 CPD. Left minus right wingbeat amplitude responses (ΔWBA) to left- and right-moving gratings were normalized and averaged so that responses in the direction of the grating were positive and responses opposing the direction of the grating were negative. For each parameter, subjects were split along the median eye area into small and large eye groups.

Contrast sensitivity, measured as the lowest discernible contrast, was not significantly affected by eye size (Figure 4 A). N=65 flies were tested and split into two bins along their median eye area of 0.11mm². The small group had a mean eye area of 0.09mm² and the large group had a mean eye area of 0.14mm². Despite these differences in eye size, corresponding to substantial differences in ommatidial size, both demonstrated a lowest discernible contrast of 0.45 (small: t(31)=5.29, p<0.01; large: t(32)=6.58, p<0.01). This corresponds to a contrast sensitivity (1/threshold Michelson contrast) of 2.22.

Based on the allometric scaling of eye area and ommatidial area measured in Section 3.1, the two eye size groups had an estimated ommatidial area of 149.3μm² for the small eyes and 191.5μm² for the large eyes. This should correspond to a 22% sacrifice in contrast sensitivity for the small eyes. The absence of any significant behavioral sacrifice in
contrast sensitivity suggests that smaller-eyed flies are using spatial or temporal summation to compensate, with implications for their spatial or temporal acuity. Spatial acuity, measured as the highest discernible spatial frequency, improved moderately with eye size (Figure 4 B). $N=45$ flies were tested and split into two bins along their median eye area of 0.14mm$^2$. The small group had a mean eye area of 0.12mm$^2$ and the large group had a mean eye area of 0.16mm$^2$. These differences in eye size, corresponding to moderate differences in inter-ommatidial angle, resulted in the larger eyes’ ability to discern spatial frequencies up to about 0.125 CPD ($t(22)=4.41$, $p<0.01$) while the smaller eyes could discern only up to 0.11 CPD ($t(21)=2.91$, $p<0.01$). This represents a 12% decrease in spatial acuity for smaller eyes.

Based on the allometric scaling of eye area and inter-ommatidial angle measured in Section 3.3, the small group had an average inter-ommatidial angle of about 4.9º while the large group had an average inter-ommatidial angle of about 4.6º. From the spatial acuity equation in Section 1.2, those inter-ommatidial angles result in spatial acuities of about 0.118 CPD for the small eyes and 0.126 for the large eyes. These estimates are very close to the behaviorally measured spatial acuities of 0.11 CPD and 0.125 CPD, suggesting that the change in spatial acuity found in smaller eyes is due to the change in optics and not spatial summation.

Temporal acuity, however, measured as the highest discernible temporal frequency, improved substantially with eye size (Figure 4 C). $N=112$ flies were tested and split into two bins along their median eye area of about 0.14mm$^2$. The small group had a mean eye area of about 0.10mm$^2$ and the large group had a mean eye area of 0.16mm$^2$. These differences in eye size corresponded to the larger eyes’ ability to discern temporal
frequencies up to about 26.3Hz (t(55)=3.07, p<0.01) while the smaller eyes could discern only up to about 10.8Hz (t(55)=4.64, p<0.01), a 59% decrease in temporal acuity for smaller eyes. Large eyes demonstrated nearly three times the temporal acuity of smaller eyes, suggesting that smaller flies are using temporal summation to achieve the same contrast sensitivity as their larger conspecifics.

4. Discussion

Optical principles dictate that, everything else being equal, a smaller eye must confer poorer vision (Land and Nilsson, 2012). Plenty of comparative studies demonstrate this in evolution, revealing optical, neural, and behavioral adaptations of small animals facing this challenge. Here we make the same case but for developmental adaptations.

4.1. Small eyes benefit by sacrificing contrast sensitivity more than spatial acuity.

Among conspecifics, small flies are at a clear optical disadvantage. Due to limited larval feeding, small flies have smaller eyes composed of fewer and smaller ommatidia, separated by slightly broader inter-ommatidial angles. The change in inter-ommatidial angle, however, is minimal compared to the change in ommatidial area and may serve to minimize the effect of diffraction. As a result, larger eyes afford almost twice the contrast sensitivity and roughly 1.2 times the spatial acuity of their smallest counterparts.

Given that a smaller eye must sacrifice at least one of the two properties – contrast sensitivity or spatial acuity – sacrificing contrast sensitivity may be the better option. While contrast sensitivity can be recovered via spatial or temporal summation, no neural process can recover spatial information once it is lost at the optical level. In real time, contrast sensitivity lost at the optical level can be recovered along one neural pathway, while high spatial or temporal information is maintained along alternative pathways (but
not both). Similarly, through development, neural summation processes might optimize to particular visual environments, increasing or decreasing spatial or temporal summation ranges within limits (which has been demonstrated at the photoreceptor level: Wolfram and Juusola, 2004). The alternative – sacrificing spatial acuity at the optical level – places an upper limit on spatial acuity and precludes any of these adaptive strategies.

4.2. Small eyes lose temporal acuity, which improves metabolic efficiency.

Despite their optical sacrifices, smaller flies demonstrate no loss in contrast sensitivity and little loss in spatial acuity based on their behavior. The loss of spatial acuity is expected from their slightly wider inter-ommatidial angles, but is too small to infer spatial summation. A much greater loss is found in the temporal acuity experiment, demonstrating a nearly three-fold loss for smaller eyes. This strongly suggests that smaller flies use temporal summation to recover the loss in contrast sensitivity due to smaller optics.

The neural activity underlying vision costs energy (Laughlin, 2001) and small flies exposed to limited larval feeding may limit their energy budget by reducing neural activity in ‘anticipation’ of a resource limited future. Metabolic efficiency, which influences the evolution and design of neural systems (Laughlin, 2001; Laughlin et al., 1998), can be improved by reducing the sensitivity or temporal acuity of photoreceptors (Laughlin, 2001; Niven et al., 2007). Interestingly, though small flies sacrifice temporal acuity, improving energy efficiency, they do not also sacrifice sensitivity. Instead, smaller flies' photoreceptors (considered separately) are likely more sensitive, and therefore more energy costly, than those of their larger counterparts to recover the sensitivity lost by their smaller lenses. Future work using intracellular measures (like Niven et al., 2007) is
needed to understand the metabolic trade-offs underlying small flies’ sacrifice of
temporal acuity over sensitivity. For instance, photoreceptor sensitivity and temporal
acuity may scale at different rates, such that developmental changes in temporal acuity
are easier than sensitivity. Ultimately, the role of energy consumption in the retina and
any underlying scaling relations trade off with visual performance (Laughlin, 2001;
Laughlin et al., 1998; Niven et al., 2007) and a complete understanding requires
examining their visual ecology and measuring both metabolic efficiency and visual
performance throughout development.

4.3. Loss of temporal acuity occurs in dark-adapted and dark-reared photoreceptors.
When adapting to dimmer environments, the photoreceptors of normally sized fruit flies,
and many other insects, increase their membrane conductance, hyperpolarizing the cell
and increasing the cell's time constant (Juusola and Hardie, 2001). This has the effect of a
low pass filter with a cutoff frequency that corresponds to ambient light levels (Juusola
and Hardie, 2001). Decreased photon capture due to a smaller lens is not fundamentally
different than that due to dim ambient light levels, so small flies might use the same
mechanism for dark adaptation to recover contrast sensitivity lost by their smaller optics.
While dark adaptation results in lower temporal acuity of the photoreceptor (Juusola and
Hardie, 2001), it is a minor decrease compared to what we found in our behavioral
experiments. Small eyes had an estimated ommatidial area of about 160μm², and large
eyes had an estimated ommatidial area of about 205μm², suggesting a decrease of about
25% of the light available to the smaller eyes' photoreceptors. This corresponded to a
decrease in temporal acuity from about 26Hz for the large eyes to about 13Hz for the
small eyes. For comparison, an average photoreceptor demonstrates a temporal frequency
cutoff of about 25Hz in ambient light of about 3X10^6 photons per second (Juusola and Hardie, 2001). After a 90% decrease in ambient light, the cutoff decreases to around 23 Hz, only 3 Hz less (Juusola and Hardie, 2001). A decrease of 10 Hz, like we found in smaller flies at the behavioral level, occurs in normal photoreceptors only after a decrease of about 99.7% in ambient light (Juusola and Hardie, 2001). However, the differences at the behavioral level may stem from smaller differences in the retina or may involve an entirely different mechanism. Future research should use intracellular electrophysiology to determine the mechanism underlying this trade-off and how it might relate to dark adaptation.

Temporal summation in smaller eyes may involve more than dark adaptation at the photoreceptor level and may be more similar to the effect of prolonged dark-rearing (Barth et al., 1997; Wolfram and Juusola, 2004). Dark rearing of normally sized flies results in a loss of as much as 30% of lamina volume (Barth et al., 1997) and an increase in time-to-peak and half-width of their voltage response of 3ms (Wolfram and Juusola, 2004). Because of their reduced light absorption, small-eyed flies may represent a less extreme case of dark rearing and future research should consider how small optics interact with dark adaptation and dark rearing.

4.4. Loss of temporal acuity has ecological implications.

Loss of temporal acuity leaves small eyes susceptible to greater motion blur than their large counterparts, making it difficult to resolve high spatial frequencies during movement. To perform comparably, small flies would have to be active in brighter environments or fly slower or further away from objects of interest. Being active in brighter environments, such as different times of the day or brighter regions, could
minimize the effects of temporal summation due to light adaptation and increase contrast sensitivity at the optical level. This, however, might isolate smaller flies or expose them, visibly, to predators they otherwise could have avoided. Alternatively, small flies might change their flight behavior to keep temporal frequencies within their visible range. Because the velocity of an image is the proportion of temporal to spatial frequencies, flight strategies that minimize the angular velocity of the image can allow the perception of higher spatial frequencies. This can be done by flying at slower speeds or further away from objects of interest. Either way, small flies must adjust their behavior or be subject to increased photon noise and reduced visual signal to noise ratio.

Temporal acuity may differ between the lateral and frontal regions of the eye. During forward motion, images in the periphery move quicker than those in front, so temporal summation would increase motion blur in the lateral regions of the eye more than in the front. Small eyes may have higher temporal acuity in the lateral regions of their eye to minimize the motion blur differences between small and large eyes. What, then, would be their peripheral contrast sensitivity? This remains unknown because we displayed gratings in the front 90 degrees of the flight arena. Interestingly, fruit flies can increase spatial summation in the periphery in response to optic flow, which attenuates the effect of motion blur (Theobald, 2017). Further research will investigate temporal processing in different regions of small versus large eyes and how this might interact with optic flow-induced spatial filtering.

4.5. Conclusion

Various factors are known to influence organ and body scaling relations in holometabolous insects (Callier and Nijhout, 2013; Shingleton et al., 2007; Shingleton et
Differences in larval nutrition, temperature, oxygen, and group density result in different organ allometries with complex interactions (Shingleton et al., 2009). The plasticity of these developmental processes implicates the often overlooked though fundamental role of the environment in establishing allometries. Many of the morphological differences between and within species, often assumed to be inherent, may actually be due to environmental conditions (Shingleton et al., 2009). Because vision depends on the absolute size of the eyes, the role of environment in the development of vision should be emphasized, especially in small animals. We have demonstrated here how limited larval feeding results in small but significant differences in eye morphology. These structural differences present a visual challenge to small flies, which was improved by temporal summation but still had substantial effects on the flies’ behavior and likely their ecology. Small flies are common in nature where environmental factors like available nutrition and temperature are variable. However, conventions of laboratory husbandry, designed to minimize variation, have obscured these ecologically relevant developmental adaptations. We find that small eyes maintain spatial acuity by sacrificing contrast sensitivity at the optical level, but recover contrast sensitivity almost completely by sacrificing temporal acuity at the neural level.

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References


Figures

Figure 1: A) Lab-reared adults that were abundantly fed as larvae (left) are generally larger than those who had limited larval food availability (right). B) Eyes are proportionate to the size of the overall body. C) Contrast sensitivity, which is limited by ommatidial diameter, $D$, and spatial acuity, inversely limited by the inter-ommatidial angle, $\Delta \phi$, must trade off. D) A computer generates visual stimuli projected onto 5 surfaces of the flight arena via 4 mirrors. E) In the flight arena, each of the fly's wingbeats are captured by an infrared light and two receivers. F) The difference in wing beat amplitudes, $\Delta WBA$, signals the fly's steering effort.
Figure 2: Body and eye sizes differ between 4 rearing conditions. In gray are lab-reared flies exposed to abundant larval feeding. In green are wild caught flies. In red are lab-reared flies exposed to restricted larval feeding. In blue are the progeny of wild-caught flies exposed to abundant larval feeding. Box plots on each axis show the range, inter-quartile range, and median for each group.
Figure 3: Smaller eyes have fewer (A) and smaller (B) ommatidia separated by wider inter-ommatidial angles (C). The scaling relations of B and C affect contrast sensitivity and spatial acuity differently (D), such that smaller eyes sacrifice contrast sensitivity (black) more than spatial acuity (green). The minimum visible wavelength of light due to diffraction is minimally affected by smaller eye size (E) due to the coordinated decrease of ommatidial area and increase of inter-ommatidial angle. Note: Filled areas represent 95% confidence bands of the mean based on the allometric (log-transformed) model.
Figure 4: Behavioral measurements of contrast sensitivity (A), spatial acuity (B), and temporal acuity (C) for large (red) and small (blue) flies. Top: plots show mean responses ± SEM taken over the last 800 milliseconds of each trial. Colored dashes below the horizontal axis signify when the mean is not significantly different from 0 using a one sample t-test at P=0.01. Bottom: time series of the mean response ± SEM for large and small flies to four select temporal frequencies demonstrate how both small and large flies can respond reliably up to 10.8 Hz, but only large flies respond reliably to 26.3 Hz. Asterisks signify when the mean during the last 800 milliseconds is significantly different from 0 using a one sample t-test at P=0.01.
CHAPTER IV
ACUITY AND SUMMATION STRATEGIES DIFFER IN VINEGAR AND DESERT FRUIT FLIES

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Abstract (190 < 250 words)

Approximately 10 thousand years ago the vinegar fly (*Drosophila melanogaster*) shifted from a single plant specialist restricted to sub-Saharan Africa into a cosmopolitan generalist, dispersing worldwide as a human commensal. Much earlier a desert fly in the same genus (*Drosophila mojavensis*) colonized the New World, specializing on rotting cactuses in southwest North America. Although desert flies differ from vinegar flies in some visual-based behaviors, little is known about their general visual capacity. The deserts they inhabit are characteristically flat, bright, and barren, implying environmental differences along multiple visual parameters. Here we demonstrate inter- and intraspecific differences in eye morphology and visual motion perception under three ambient light levels. A reduction in ambient light from 222 to 111 lux causes sensitivity loss in desert flies, but vinegar flies neurally summate to avoid this. However, at 20 lux desert flies summate more than vinegar flies, sacrificing spatial and temporal acuity more severely to maintain contrast sensitivity. These visual differences help the desert fly navigate in the harsh desert while accommodating their crepuscular and biphasic adult lifestyle. Vinegar flies’ more sensitive but less severe light adaptation strategy allows them to see under wide-ranging light levels around the world.
The vinegar fly (*Drosophila melanogaster*; Figure 1 A, left column) buzzing around your ripe fruit or open beer, is surprisingly widespread (Markow 2015). Once mostly restricted to sub-Saharan Africa (Throckmorton 1975) where they used rotting fruit, likely marula, as their host (Karageorgi, Matsunaga, and Whiteman 2018; Mansourian et al. 2018; Markow 2015), they dispersed with humans to Asia and Europe around 10 thousand years ago, and then to Australia and the Americas within the past few centuries (David and Capy 1988; Markow 2015). As a human commensal, they shifted to a cosmopolitan generalist lifestyle, targeting fruits around the world (Atkinson and Shorrocks 1977; Karageorgi et al. 2018; Mansourian et al. 2018). However, the *Drosophila Siphlodora* subgenus (Yassin 2013) beat vinegar flies to the New World by about 30 million years, radiating first in South America (O’Grady and DeSalle 2018; Smith et al. 2012; Throckmorton 1975). Members of what would become the *repleta* species group shifted to feeding and breeding on fermenting cactuses, likely from arid regions of Peru and Bolivia (Smith et al. 2012), and many followed cactus populations dispersing northward. One cactophilic fly (*D. mojavensis*, Figure 1 A, middle and right columns) colonized deserts of southwest North America, diverging from Mexico over the past ~300,000 years into subspecies specializing on various endemic cactuses (Mojave, Sonoran, and Baja California; Smith et al. 2012). Because 1) their ecologies are unique and include a history of host shifts, geographic isolation, chromosomal inversions, and incipient speciation, 2) the *repleta* group is monophyletic, and, 3) among other cactophilic species, the desert fly has had its genome sequenced and shared publicly (Drosophila 12 Genomes Consortium 2007), cactophilic flies are a model system for studying genetics, ecology, speciation, and their interactions (Markow 2019; Smith et al. 2012).
Although their eyes are superficially similar, profound ecological differences have driven vinegar and desert flies to see differently. Desert flies have larger eyes absolutely, and relative to their antennae (Keesey et al. 2019). Vinegar flies correct for visual sideslip above and below their head, but desert flies suppress responses to all sideslip above and some below, responding specifically to the low visual clutter expected in barren deserts (Ruiz 2021). Vinegar flies also steer away from small visual spots (Maimon, Straw, and Dickinson 2008; Park and Wasserman 2018), which may help avoid predators (Maimon et al. 2008), but this response is absent in desert flies (Park and Wasserman 2018).

However, little is known about the general visual capacity of desert flies. Given that visual ecologies and responses both differ, what visual traits adapt desert flies to the unique desert landscape, in contrast to the cosmopolitan vinegar fly?

_Drosophila_ wield neural superposition eyes of about 800 facets each (Figure 1 B, insets). Each facet, called an ommatidium, isolates light from neighbors by pigment cells, and directs light onto photoreceptors with a lens and crystalline cone (Ready, Hanson, and Benzer 1976). Lens size limits light collection and thus, partially, the ability to differentiate brightness levels, which is critical when under dim light or during fast image motion (Snyder, Laughlin, and Stavenga 1977; Snyder, Stavenga, and Laughlin 1977; Theobald 2017; Warrant 1999). The angular separation of ommatidia, called the interommatidial (IO) angle, limits the smallest discernible details and thus inversely limits resolution of spatial detail, called spatial acuity. Acuity is necessary for object recognition, collision avoidance, and perceiving small changes in self-motion (Land 1997; Land and Nilsson 2012).
Because *Drosophila* eyes are approximately spherical, the IO angle is safely approximated as $\Delta \varphi = D / R$, where $\Delta \varphi$ is the IO angle, $D$ the ommatidial diameter, and $R$ the eye radius (Figure 1 C; Land 1997; Stavenga 1979). The total visual angle defines the field of view (*FOV* in Figure 1 C), the sum of IO angles subtended. This geometric relation demonstrates a special case of how visual acuity and sensitivity trade off (Land and Nilsson 2012; Snyder, Laughlin, et al. 1977): for a given $R$ and *FOV*, increasing sensitivity by increasing $D$ decreases acuity by decreasing $1 / \Delta \varphi$, and vice versa. Likewise, if *FOV* remains constant and $R$ decreases, the eye must sacrifice 1) spatial acuity by decreasing $1 / \Delta \varphi$ and consequently the number of ommatidia, 2) contrast sensitivity by decreasing $D$, or 3) both. It is therefore illuminating to determine how parameters change with eye size (Shingleton et al. 2007; Voje et al. 2014), such as in small vinegar flies that sacrifice optical sensitivity more than acuity (Currea, Smith, and Theobald 2018). Should the same hold for desert flies?

Deserts are often flat, open-country habitats where predators or cactuses adhere to a planar surface. Inhabitants of flat environments frequently possess wide *FOVs* and enhanced acuity along the horizontal meridian (the terrain theory, Hughes 1977), found among mammals, birds, reptiles, amphibians, and fish (Collin and Pettigrew 1988; Johnson and Gadow 1901; Luck 1965; Potier, Mitkus, and Kelber 2020; Pumphrey 1948; Wood 1917; for examples, see Hughes 1977), and fiddler crabs (Brodrick et al. 2020; Smolka and Hemmi 2009) backswimmers (*Notonecta*), waterstriders (*Gerridae*), and some empidid flies (Zeil, Nalbach, and Nalbach 1989). These are likely adaptations to horizons with few interruptions, not just open-country (Hughes 1977), and seem absent in nocturnal open-country dwellers (Hughes 1977; Lisney et al. 2012). And although the
Saharan *Cataglyphis* ants have a horizontal streak of increased acuity (Wehner, Cheng, and Cruse 2014), Australian desert ants (*Melophorus bagoti*) have a streak of increased sensitivity and reduced acuity (Schwarz, Narendra, and Zeil 2011), reflecting differences in lifestyle or clutter typical to each desert (Wehner et al. 2014). Evolution of the wide FOV and horizontal streak therefore depends on a visible horizon largely uninterrupted by vegetation or darkness, and the nature of the streak may depend on lifestyle or habitat image statistics.

Deserts often lack shade or terrain relief, and so provide unique tradeoffs for sensitivity and acuity (Land 1997; Snyder, Laughlin, et al. 1977; Snyder, Stavenga, et al. 1977). $D$ and $\Delta \phi$ (Figure 1 A) are limited by diffraction through ommatidia, and the random nature of light absorption (Snyder, Stavenga, et al. 1977), and maximizing spatial information requires an eye at the diffraction limit, with a maximum discernible wavelength of $\lambda_{\text{max}} < 2D \cdot \Delta \phi$ (Howard and Snyder 1983; Snyder 1979). Without a canopy, deserts are generally bright (Schwegmann, Lindemann, and Egelhaaf 2014), likely favoring smaller ommatidial diameters (lower sensitivity) and narrower IO angles (higher acuity), with $\lambda_{\text{max}}$ closer to the upper limit of spectral sensitivity. Thus, small conspecifics may sacrifice sensitivity to maintain competitive levels of horizontal FOV and spatial acuity.

However, both desert and vinegar flies are active at sunrise and sunset (Hardeland and Stange 1973). To improve sensitivity in dim light, animals can neurally summate over time or angular space (Snyder, Stavenga, et al. 1977; Warrant 1999). Temporal summation collects photons for longer durations, similar to a long photographic exposure, improving sensitivity at the cost of high temporal resolution (Warrant 1999). Vinegar flies temporally summate in dim light (Juusola and Hardie 2001;
Palavalli-Nettimi and Theobald 2020) and smaller flies temporally summate to achieve the sensitivity of larger ones (Currea et al. 2018). Spatial summation increases the effective IO angle, similar to using grainy photographic film, improving sensitivity at the cost of high spatial resolution (Snyder 1979; Warrant 1999). This is a common response to low light or fast image velocity (Theobald, Warrant, and O’Carroll 2010; Warrant 2017). Vinegar flies spatially summate in the dark (Palavalli-Nettimi and Theobald 2020) and facultatively during forward optical flow (Theobald 2017). Further, canopies substantially change image statistics (Dyakova et al. 2019; Schwegmann et al. 2014), creating variable levels of brightness—from heavy shade to direct sunlight. Canopy shadows in forest reduce brightness and increase spatial contrast compared to an open field (Dyakova et al. 2019; Schwegmann et al. 2014). A foraging vinegar fly thus faces a range of brightness, possibly requiring summation over short flight distances. A desert fly faces infrequent changes in brightness, lingering around a cactus, hiding in the shadow (Krebs and Bean 1991). A necrotic cactus eventually rots out, leaving desert flies in search of another host under no canopy, requiring light adaptation that shows less sensitivity to small changes (Dyakova et al. 2019; Schwegmann et al. 2014), but still accommodating a crepuscular lifestyle.

Because many deserts are characteristically flat, bright, and barren, we offer several predictions about desert compared to vinegar flies: a wider FOV and horizontal streak, greater spatial acuity and lower contrast sensitivity, and a less sensitive but accommodating light adaptation strategy. Also as eye size decreases, we predict that desert flies sacrifice sensitivity more than vinegar flies to maintain horizontal FOV, spatial acuity, or both. Here we test these predictions using a combination of microscopy,
allometry, optical modeling, and virtual reality flight simulation (Figure 1D–E and Figure 2). In the process, we demonstrate several visual differences that help the desert fly survive in the harsh deserts of southwest North America, while enabling vinegar flies to thrive under wide-ranging light levels the world over.

Results

General Allometry: Desert Flies Have Larger Eyes

To characterize general morphological differences between the two species, we compared measurements of abdomen, thorax, head and eye length and their allometries in relation to body length (Figure 3). Mean differences were assessed by pairwise T tests using Šidák-Holm corrected p-values and indicated in the box plot comparisons of Figure 3. Relevant parameters from the allometric regressions are found in Supplementary Table 1. We compared the vinegar fly to two desert fly subspecies: *D. mojavensis baja* from the Baja California Desert, which uses agria cactus (*Stenocereus gummosus*) as host; and *D. mojavensis mojavensis* from the Mojave Desert, which uses barrel cactus (*Ferocactus cylindraceus*; Smith et al. 2012). Despite unique host preferences, we reared all three on the same instant *Drosophila* medium. *D. mojavensis baja* (hereafter referred to as *D. moj baja*) had a longer body, abdomen, and thorax than *D. mojavensis mojavensis* (*D. moj moj*), with *D. melanogaster* (*D. mel*) generally in the middle (Figure 3 A–B). Abdomen and thorax lengths shared positive allometries with body length for all three flies, with largely overlapping confidence intervals (CIs) for the allometric constant, *b* (Figure 3 A–B). *b* represents the growth rate of one trait—here abdomen or thorax length—with respect to a reference trait like body length.
The relationships change for head and eye length. For the head, though *D. moj baja* was again longer than *D. moj moj*, *D. mel* was shorter than either desert species (Figure 3 C). Also, while allometric regressions for desert flies were similar and represented the data well, in *D. mel* neither the allometric regression nor the resulting allometric constant were statistically significant. So in desert flies head length was related to body length and followed the same hypoallometry, but it had no such relation in *D. mel*.

In eye length, *D. moj baja* was greater than both *D. mel* and *D. moj moj*, with no significant difference between *D. moj moj* and *D. mel* (p = .02; Figure 3 D). In contrast to head length, eye length followed similar positive hypoallometries for all three flies. Nonetheless, because *D. mel* had mid-range body lengths, they had shorter eyes relative to body length (0.169 ± 0.012; not graphed) than *D. moj moj* (0.184 ± 0.010; p < .001) but not *D. moj baja* (0.175 ± 0.012; p = .08), with *D. moj baja* less than *D. moj moj* (p < .05).

Therefore, while the desert flies followed nearly equivalent positive allometries for each trait, *D. mel* did so with all but head length, which was unrelated to body length. Because head length corresponded greatly with eye width, the positive allometries suggest desert species have wider eyes that increase in width as a function of body length, while *D. mel* maintain constant eye width. Altogether, desert flies have larger eyes, and in *D. moj moj*, larger proportional to body length.

**Eye Allometry**

**Eye Shape and FOV: Desert Flies Have A Broader and Particularly Wider FOV**

To characterize general differences in eye structure and visual field, we compared measurements of eye area, eye radius, and vertical and horizontal components of FOV.
(Figure 1 and 4). As predicted, *D. mel* had eyes with lower surface area than desert flies, and no significant difference within desert flies (p = .08; Figure 4, horizontal axis and box plots). Conversely, eye radius showed no significant differences (Figure 4 A, p > .989) and all three shared similar positive hypoallometries. In vertical FOV (Figure 4 B), *D. mel* were more restricted than desert flies, with no significant difference within desert subspecies (p = .75). Vertical FOV was largely independent of eye size for all flies and each allometric regression was a poor representation of the data, suggesting no significant allometries. In horizontal FOV (Figure 4 C), *D. mel* were again more restricted than the desert flies. However, the 13° difference between *D. mel* and *D. moj moj* in horizontal FOV was more than twice the 6° difference in vertical. This is apparent in dorsal head images, revealing that desert fly eyes recede further back into the thorax and wrap around further than vinegar flies (Figure 4 G). The desert flies, especially *D. moj baja*, also appear to have shorter necks and likely restricted head movement. Horizontal FOV was largely independent of eye size and each allometric regression was a poor representation of the data, suggesting no significant allometries. We further analyzed vertical-to-horizontal FOV ratio (not plotted). *D. mel*, had a longer eye (1.31 ± 0.05) than desert flies, *D. moj baja* (1.24 ± 0.05; p < .001) and *D. moj moj* (1.19 ± 0.05; p ≪ .001), with *D. moj moj* less than *D. moj baja* (p ≪ .001).

In summary, the desert flies had larger eyes with the same average eye radius as *D. mel*. Eye radius increased by similar functions of eye size for all three, implying that *D. mel* had the largest radius relative to eye size. As a result, desert flies had greater, especially wider FOVs, which remained relatively constant with eye size. This confirmed the
prediction of wider eyes based on general difference in head and eye length (Figure 3 C–D), though found no significant FOV allometry in the desert flies. Instead, each had a characteristic V/H FOV ratio independent of eye size, with the desert flies, especially *D. moj moj*, closer to 1.

**Eye Optics Approximations: Desert Flies Have More Acute But Less Sensitive Eyes**

We further analyzed ommatidia distribution across the visual field to approximate parameters of optical performance. In numbers (Figure 4 D), *D. moj baja* had ~200 more ommatidia than *D. moj moj* and ~450 more than *D. mel*, with ~250 more in *D. moj moj* than *D. mel*. The allometric regression for each was a good representation of the data. The regressions had generally overlapping CIs for *b*. As with absolute ommatidia counts, ommatidal density (ommatidia count / eye area; not plotted) was lower in *D. mel* (4300 ± 250 mm⁻²) than in desert flies, *D. moj moj* (4740 ± 420 mm⁻²; *p* < .001) and *D. moj baja* (6020 ± 530 mm⁻²; *p* ≪ .001), with *D. moj moj* less than *D. moj baja* (*p* ≪ .001).

In IO angle (Figure 4 E), the desert flies were narrower than *D. mel*, with *D. moj moj* narrower than *D. moj baja*. IO angle followed a significant negative allometry with eye size in *D. mel* but not in desert flies. Conversely, in ommatidial lens areas (Figure 4 F), *D. mel* were larger than the desert flies, with *D. moj baja* smaller than in *D. moj moj*. Lens area followed similar hypoallometries for all three with overlapping CIs for the allometric constant, though it was insignificant in *D. moj baja*.

As mentioned earlier, IO angle and lens area set limitations on spatial acuity and sensitivity. Because desert flies have narrower IO angles but smaller lenses, they have the capacity for greater acuity (but at lower sensitivity) than *D. mel*. Although lens area correlated positively with eye size in all flies (Figure 4 F), IO angle was only negatively
correlated within *D. mel*. Consequently, as eye size increases, all flies improve sensitivity, but only *D. mel* improves acuity, by reducing IO angle and thus the smallest discernible details.

Finally, the maximum discernible wavelength due to diffraction (not plotted) was greater in *D. mel* (1680 ± 66 nm) than the desert flies, *D. moj baja* (1081 ± 262 nm; *p* ≪ .001) and *D. moj moj* (1293 ± 121 nm; *p* < .001). Further, neither the allometric regression nor the resulting allometric constants were significant (*p* > .09). As a result, the effect of diffraction was independent of eye size and the eyes of the desert species were 64–77% closer to the diffraction limit than *D. mel*, assuming equivalent spectral sensitivities, as expected for the visual requirements of the amply lit desert.

**Psychophysics**

*Contrast Sensitivity: Desert Flies < Vinegar Flies, Except in Low Light*

Since *D. moj baja* resisted periods in the flight arena, we compared only *D. mel* to *D. moj moj*, which had smaller but significant differences in IO angle and ommatidial area. Nonetheless, both species had the same contrast sensitivity (the inverse of the threshold contrast) of .094⁻¹ = 10.6 when the room lights were on and the projector was at full brightness (Figure 5, middle column). Actually, *D. moj moj* had significantly greater steering responses for 4 of the 6 contrasts in the range .15–.70, suggesting a small sensitivity advantage. With a neutral density filter applied to the projector, reducing the projector brightness by ~90% (Figure 5, left column), contrast sensitivity in *D. mel* remained the same while in *D. moj moj* it dropped to 5.3. Finally, with lights off and the projector at full brightness (Figure 5, right column), responses changed dramatically for both species, dropping to 3.1 in *D. moj moj* and 1.8 in *D. mel*.
These results were curious. Given their smaller ommatidia, how did *D. moj moj* achieve contrast sensitivity equal to or even greater than *D. mel* when ambient light was low? And, given the ~90% reduction in projection brightness, how did *D. mel* avoid losing sensitivity when the filter was applied to the projector?

**Spatial Acuity: Desert Flies > Vinegar Flies, Except in Low Light**

As expected, spatial acuity differed between species across all three lighting conditions, though the difference inverted under low light. With room lights on and the projector at full brightness (Figure 6, middle column), *D. moj moj* had a spatial acuity of 0.13 cycles per degree (CPD) while *D. mel* had a spatial acuity of 0.10 CPD, with *D. moj moj* greater than *D. mel* for spatial frequencies from 0.04 to 0.10 CPD. With the filter applied to the projector (Figure 6, left column), *D. moj moj* kept the same spatial acuity of 0.13 CPD while *D. mel* dropped to 0.08 CPD, with *D. moj moj* greater than *D. mel* for spatial frequencies from .05 to .13 CPD. So, *D. moj moj* had a greater spatial acuity when ambient lights were on, which is consistent with the smaller IO angles we found in *D. moj moj*. However, with lights off and the projector at full brightness (Figure 6, right column), spatial acuity in *D. moj moj* reduced to 0.08 CPD, while in *D. mel* it returned to their maximum acuity of 0.10 CPD. Likewise, *D. moj moj* responded greater than *D. mel* to low spatial frequencies up to 0.05 CPD while *D. mel* responded greater than *D. moj moj* at 0.10 CPD.

**Temporal Acuity: Desert Flies > Vinegar Flies, Except in Low Light**

The species also modulated temporal acuity differently under the different lighting conditions. Here, results based on measured differences of the maximum detectable temporal frequency were confounding. Instead, temporal acuity was defined by the
frequency preceding a >50% drop in ΔWBA, analogous to its half-power point.

Inferences based solely on statistical significance would ignore the large drop in contrast caused by inadequate temporal acuity. With room lights on and projector at full brightness (Figure 7, middle column), both D. moj moj and D. mel had a temporal acuity of 20 Hz. Actually, D. moj moj responded greater than D. mel at 20 Hz and D. mel more than D. moj moj at frequencies less than 5 Hz, suggesting a small temporal acuity advantage for D. moj moj. With the filter (Figure 7, left column), D. moj moj showed no measurable change in responses while D. mel reduced responses to 20 Hz by >50%, implying a temporal acuity reduction to 10 Hz. Further, D. mel responses were stronger at 5 Hz while D. moj moj responses were stronger at 20 Hz implying different optima.

Finally, with lights off and the projector at full brightness (Figure 7, right column), D. mel maintained a temporal acuity of 10 Hz while D. moj moj reduced ΔWBA by >50% in response to 20 Hz, implying a temporal acuity reduction to 10 Hz.

Light Adaptation: Sensitive vs. Severe

Ambient light and projection brightness had different effects on the contrast sensitivity and temporal and spatial acuity of the two species. For D. mel, reducing projector brightness—lowering ambient light from 222 to 111 lux—reduced spatial acuity by a relative change (\(\frac{1-0.93}{0.1}\)) of 17% (Figure 6, top row) and temporal acuity by 50% (Figure 7, top row) but had no measured effect on contrast sensitivity. Then, when ambient lights were off—lowering ambient light to 20 lux—spatial and temporal acuity remained the same (Figures 6 and 7, top row) but contrast sensitivity reduced by 83% (Figure 5, top row). Conversely for D. moj moj, reducing ambient light from 222 to 111 lux reduced contrast sensitivity by 50% (Figure 5, bottom row) but both spatial and temporal acuity
remained constant (Figures 6 and 7, bottom row). However, reducing ambient light to 20 lux reduced spatial acuity by 33%, temporal acuity by 50%, and contrast sensitivity by an additional 42% (or 71% of the maximum). Thus, light adaptation in *D. mel* was more sensitive to small brightness changes while in *D. moj moj* spatial and temporal summation were used more severely but only after a large reduction in ambient light.

**Discussion**

These observations resolve many of our earlier predictions. First, the terrain theory posits that animals from open-country terrains evolve a wide FOV and horizontal streak of increased acuity (Hughes 1977). While we found no evidence for the horizontal streak (see zoomed insets in Figure 1 B), desert flies have a larger eye with a particularly wider FOV, corroborating that desert flies allocate more resources to vision than vinegar flies (Keesey et al. 2019) and satisfy the FOV prediction of terrain theory. Interestingly, neither horizontal nor vertical FOV followed an allometric relation to body size within species, suggesting that FOV faces strong but different selective pressures for both flies (Wehner et al. 2014). As mentioned earlier, evolution of the visual streak depends on a visible horizon, and as both flies are crepuscular, the effect may be weak, or the divergence time (~40 MY) too short.

Instead, the streak could manifest neurally by differential spatial summation across the eye. In fact, vertebrate visual streaks result from spatial pooling of retinal ganglion cells (Hughes 1977). Similar regional spatial summation would be externally invisible, and could be facultative (Theobald 2017), making regional acuity sacrifices only when needed, such as during foraging but not courtship. Alternatively, like Australian desert ants (*Melophorus bagoti*; Schwarz et al. 2011), desert flies might be an exception to
terrain theory, based on unknown environmental factors or lifestyle. Future work could measure acuity differences across the visual field to test for neural implementations of horizontal streaks.

Next, because deserts are characteristically bright, we predicted desert flies’ eyes are less sensitive, more acute, and closer to the diffraction limit (Snyder 1977, 1979). These were confirmed with eye morphology, revealing smaller ommatidia separated by narrower IO angles in desert flies than vinegar flies, and implying a lower maximum discernible wavelength. Further, while vinegar flies sacrifice both acuity and sensitivity with decreasing eye size, desert flies sacrifice only sensitivity. We also predicted that small desert fly conspecifics may sacrifice sensitivity ($D$ in Figure 1 C) more than vinegar flies in order to minimize losses in acuity ($1 / Δφ$) and FOV. Though the differences were not significant, higher allometric constants in desert flies ($D. moj moj$: $b = .6$, $D. moj baja$: $b = .8$) than vinegar flies ($b = .3$) would imply more extreme losses in sensitivity for small desert flies, in line with geometric predictions. Some of these observations were consistent with optomotor behavior in the flight arena, where $D. moj moj$ demonstrated higher spatial and temporal acuities but lower contrast sensitivity under moderate light (111–222 lux). Because both species are active at similar times (Hardeland and Stange 1973; Keesey et al. 2019), this may be due to the lack of shade from vegetation or terrain relief in many deserts.

Fewer desert shadows generate a less immediate brightness range than habitats of the cosmopolitan vinegar fly, but both flies are crepuscular, requiring sensitivity to the large, gradually changing brightness range of sunrise and sunset. We predicted desert fly vision is less susceptible to small changes in ambient brightness but still responsive to large
changes, and tested this by performing flight arena experiments under 3 different light levels (20, 111, and 222 lux). We found desert flies make greater spatial and temporal acuity sacrifices than vinegar flies to maintain contrast sensitivity only after a large reduction in ambient light (from 111 to 20 lux). This strategy of less sensitive but more severe summation affords desert flies high acuity under moderate light, like when foraging for a cactus on the horizon, but permits adequate sensitivity when light is sparse, like during courtship, which occurs in the early morning shadow of a host cactus (Krebs and Bean 1991). Future work could measure natural light levels during behaviors like foraging, as little is known about how desert flies forage for new cactus hosts.

In addition to comparing vinegar and desert flies, we measured morphological differences between desert subspecies. When reared on standard media, both D. moj moj and D. moj baja follow nearly equivalent allometries for body and thorax length, though D. moj baja are generally larger. Because D. moj baja are smaller than the Sonoran Desert subspecies (D. mojavensis sonorensis; Etges and Heed 1987), D. moj moj is smaller than at least 2 of the 3 known D. mojavensis subspecies. Still, D. moj moj and D. moj baja eyes are similar in size and allometries for surface area, radius, and vertical and horizontal FOV. Yet D. moj moj eyes contain fewer, larger ommatidia separated by broader IO angles than D. moj baja, suggesting D. moj moj have greater contrast sensitivity and D. moj baja have greater spatial acuity. These likely correspond to differences in cellular proliferation and growth during the formation of the eye-antennal imaginal discs (Currea et al. 2018).

For desert flies, the necrosis chemistry of different cactus species differentially affects gene expression (Matzkin et al. 2006), development time, viability, and overall size (Etges and Heed 1987). So, because D. moj baja target the Pitaya agria cactus
(Stenocereus gummosus) and D. moj moj target California barrel cactus (Ferocactus cylindraceus; Smith et al. 2012), but we reared both on standard Drosophila media, the ecological validity of these comparisons is speculative. Cactus stem diameter affects desiccation and correlates with total rot duration and subsequently thorax size across many cactophilic species including D. moj, and between the subspecies of D. moj baja and D. mojavensis sonorensis (Etges and Heed 1987). D. mojavensis sonorensis are larger than both D. moj moj and D. moj baja and use organ pipe cactus (Stenocereus thurberi) which have a mid-range stem diameter of 5–20 cm (Anderson 2001). So, if our results reflect natural size distributions, then D. mojavensis are an exception to this positive correlation between stem diameter and body size: California barrel cactus is ≤50 cm in stem diameter but hosts the smaller D. moj moj while Pitaya agria cactus is 3–6 cm but hosts the larger D. moj baja (Anderson 2001; Smith et al. 2012). Future work should investigate how specific resources like medium composition and temperature, which influence allometry in holometabolous insects (Callier and Nijhout 2013; Shingleton et al. 2007; Stern and Emlen 1999) and cactophilic flies in particular (Etges and Heed 1987; Etges and Klassen 1989), affect eye development and subsequently visual performance across desert flies.

**Conclusion**

More work will clarify the particular evolution of these visual traits. For instance, are desert fly traits ancestral and lost by the unique evolution of the vinegar fly, or a unique adaptation to the desert, as suggested by the terrain hypothesis? The D. repleta species group offers a unique system for probing these questions because D. mojavensis and the other North American repleta species diverged from South American relatives about 12
MY ago (Markow 2019; Smith et al. 2012). The natural replication of cactophilic flies in the Americas—such as *D. buzzatii* which uses prickly pear cactus (*Opuntia*) as host—offers a quasi-experiment in the evolution of these traits. In particular, the *D. mojavensis* and *D. buzzatii* genomes have been sequenced (Guillén, Casillas, and Ruiz 2019; Markow 2019), allowing for advanced methods of genetic analyses and maybe neurogenetic techniques to compare underlying neural processes. Despite these unknowns, desert flies certainly have larger eyes, a wider FOV, and more, smaller ommatidia than vinegar flies, fitting several predictions based on characteristics of their desert environment. Due to the critical role of neural summation for light adaptation, inferences based on eye morphology depend on features of the visual environment, and our results highlight the importance of studying behavior across different ambient light levels. In doing so, we found visual differences that help desert flies survive in the flat, bright, and barren desert while accommodating their crepuscular and biphasic adult lifestyle. Meanwhile, vinegar flies’ more sensitive but less severe light adaptation allows them to see under wide-ranging light levels around the world.

**Author Contributions**

JC: Conceptualization, Methodology, Software, Visualization, Writing- Original Draft, and Data Collection and Curation.

RF: Writing- Review and Editing, and Data Collection.

SW: Conceptualization, Methodology, Writing- Review and Editing, Supervision, Funding acquisition.

JT: Conceptualization, Methodology, Writing- Review and Editing, and Funding acquisition.
**Funding**

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**Acknowledgements**

We thank members of the Theobald Lab at FIU, especially Carlos Ruiz for his expertise in fly natural history and his excellent photography.

**STAR Methods**

**Subjects**

*Drosophila melanogaster* (Meigen) were drawn from a colony reared in laboratory conditions for several years. *D. mojavensis mojavensis* and *D. mojavensis baja* were sent from the Garrity Lab at Brandeis University and subsequently raised under the same protocols. For all experiments, wild-type flies from the three genotypes were reared at 21.5°C, under a 12 hour light:12 hour dark cycle, and on Carolina Formula 4-24 Instant *Drosophila* Medium, Plain. Individuals were randomly selected for each genotype.

**Allometric Model**

The scaling relation between physical traits, called allometry, helps quantify environmental reaction norms. They are usually modeled as a power law, \( Y = aX^b \), where \( Y \) and \( X \) are traits and \( a \) and \( b \) are constants (Shingleton et al. 2007; Voje et al. 2014). The allometric constant, \( b \), represents the growth rate of \( Y \) with respect to \( X \). Given \( a > 0 \), \( b = 0 \) implies that \( Y \) is constant with respect to \( X \); \( 0 < b < 1 \) implies hypoallometry, so that as \( X \) increases, \( Y \) increases at a decreasing rate; \( b = 1 \) implies isometry or linear scaling.
between $X$ and $Y$; and $b > 1$ implies hyperallometry, so that as $X$ increases, $Y$ increases at an increasing rate. $a < 0$ implies the same relations except with an inverse allometry.

To apply this model, we log transformed both traits $X$ and $Y$ and then used ordinary least squares regression to model $\log(Y)$ as a function of $\log(X)$,

$$\log(Y) = \log(a) + b \cdot \log(X).$$

84% confidence bands, used for comparing parameter means between species with $a = .05$ (Goldstein and Healy 1995), were approximated by applying the regression model in log space and then exponentiating $e$ to the power of the resulting estimates (shaded areas in Figures 3 and 4, for example).

**General Allometry**

Subjects were drawn from each genotype 3–6 days post eclosion and were euthanized by placing them in a freezer for 48 hours. Next, they were glued to a rigid tungsten rod, 0.02 mm in diameter, on the dorsal prothorax for easy manipulation and were photographed laterally using a digital recording microscope (Zeiss Axio Scope.A1). Trait lengths were measured manually on the microscope images using a custom Python program (Figure 1 A). Eye length was measured as the longest line through the eye, $ab$, which was approximately vertical and perpendicular to the head length. To account for idiosyncratic bending of the abdomen or head, body length was measured as the sum of the lengths of the three body segments. The head segment was measured from the dorso-anterior tip of the antenna flagellum, $c$, to the anterior tip of the head occiput, near the center of the neck and often coinciding with anterior tip of the eye, $d$. The thorax segment was measured from $d$ to the base of the haltere, $e$. The abdomen segment was measured from $E$ to the posterior tip of the abdomen, $d$. The more conventional thorax length was taken from the anterior margin at the neck ($g$) to the posterior tip of the scutellum ($h$; Atkinson and Sibly
We analyzed how abdomen (ef), thorax (gh), head (cd), and eye lengths (ab) scale with respect to body length (cdef; Figure 1 A). The values needed to compare these allometries are available in Supplementary Table 1.

**Eye Allometry**

For eye measurements, live adults were drawn from the 3 genotypes around 3-6 days post eclosion and were cold-anesthetized and glued to a rigid tungsten rod, 0.02 mm in diameter, on the dorsal prothorax. Flies from *D. melanogaster* and *D. mojavensis* were then placed in the flight arena for vision experiments described below. Flies from *D. mojavensis baja* proved too difficult to test in the arena and were excluded from our behavioral experiments.

Next, glue was applied to the neck, adhering the head to the thorax to avoid head motion during imaging. Using the digital recording microscope, multiple images (~20) were taken per fly from one angle, at fixed intervals of focus depth (Figure 2 A). Using measurements of local contrast, we can measure for each pixel which layer—and thus height—the eye surface is in greatest focus, allowing us to reconstruct the 3D eye surface (Figure 2 B). Eye radius was calculated by finding the best fitting sphere to the 3D eye surface (Figure 2 D). Using the center of the sphere, the coordinates were spherically transformed, allowing us to generate a flattened image of the eye correcting its curvature (Figure 2 D). FOV was calculated as the area of a best fitting ellipse to the outline of the eye in spherical coordinates and its vertical and horizontal components as the major and minor diameters. Note that distance and area in spherical coordinates correspond to angle and solid angle in cartesian coordinates.
To measure optically relevant parameters, an open source Python program (Currea et al. 2021) generated a single image composite of the stack of photos (Figure 2 A, bottom). Ommatidia counts and diameters were measured using the ommatidia detecting algorithm (Currea et al. 2021). IO angle was calculated as $\Delta \phi = D/R$, where $D$ is the ommatidial diameter and $R$ the eye radius. Ommatidial area, which as opposed to diameter is directly proportional to optical sensitivity, was measured as the area of a circle defined by the ommatidial diameter, $A = \pi \left( \frac{D}{2} \right)^2$. Finally, the maximum discernible wavelength was calculated as $\lambda < 2D \cdot \Delta \phi$, assuming that lens acuity is strictly less than spatial acuity (Howard and Snyder 1983; Snyder 1979). The values needed to compare these allometries are available in Supplementary Table 2.

**Psychophysics**

**Flight Arena**

The flight arena consisted of a back-projection lined perspex cube with four first-surface mirrors angled to project a stimulus onto five of its six sides (Figure M1). For more details see (Cabrera and Theobald 2013). The current study used only the front $\pm 45^\circ$ azimuth and $\pm 45^\circ$ or $\pm 67.5^\circ$ elevation, mostly occupying the front 90 X 90°, 229 X 229 pixel panel. At the center of the arena, each tethered fly tries to minimize the perception of motion, but is left immobilized while still beating its wings (Götz 1987; Tammero, Frye, and Dickinson 2004). An infrared LED, which is invisible to and placed above the fly, casts a shadow of each wing onto a corresponding photodiode below. The photodiodes provide a time series of the fly's left and right wing beat amplitude ($\Delta$WBA) at 1 kHz. The difference between the left and right $\Delta$WBA indicates the fly's perceived direction of motion and is proportional to yaw torque (Figure M1; data sample; Götz,
1987; Tammero et al., 2004). For all experiments using the flight arena, ΔWBA was normalized to the maximum of each lighting condition, species, and experiment so that it corresponds to a proportion of the maximum response in that specific condition.

**Lighting Conditions**

Flight arena experiments were conducted under three different lighting conditions. Initially, flies were tested in a dark room, with the lights off. This resulted in a maximum Michelson contrast of 98% and an average brightness of 20 lux inside the arena. Later, a different sample of flies was tested in a room with the lights on and with or without a neutral-density filter applied to the projector. The neutral-density filter reduced the brightness of the projection by roughly an order of magnitude (~90%). Having the room lights on resulted in a maximum Michelson contrast of 85% and an average brightness of 111 lux inside the arena with the filter applied to the projector and 222 lux without the filter.

**Gratings**

In the arena, flies were presented a series of experiments to measure the visual consequences of their eye structure. Experiments consisted of open-loop sequences of moving sinusoidal gratings interspersed by 3 s bouts of closed-loop fixation of a vertical striped bar. During fixation, the fly controlled the position of the striped bar, improving their responsiveness to experimental stimuli (Heisenberg and Wolf 1979; Reichardt and Wenking 1969). During test sequences, grayscale sinusoidal moving gratings from a list of spatial frequencies, temporal frequencies, and contrasts, moving either to the left or right, were presented in a randomized order. Each grating was presented for about 3 s, followed by the fixation task, until each fly was exposed to the whole list. For
experiments with lights on, whenever possible, flies were randomly assigned to view all experiments in one projector condition followed by the opposite.

The moving sinusoidal grating is ideal for measuring optical performance. It allows the independent manipulation of contrast, spatial frequency, and temporal frequency while maintaining the same mean luminance across all conditions. Contrast is defined as the Michelson contrast, which is the difference between the brightest and darkest intensities of the grating divided by their sum. Contrast sensitivity is measured as the inverse of the lowest detected contrast. Spatial frequency defines the frequency of luminance change over distance in cycles / ° (CPD). Spatial acuity is measured as the highest detected spatial frequency (Land 1997; Land and Nilsson 2012). Temporal frequency defines the frequency of luminance change over time in cycles per second or Hz. Though temporal acuity is often measured as the highest detected temporal frequency, an accurate and complete description of acuity includes relative ability to respond at different frequencies. Here temporal acuity is defined as the temporal frequency that follows a significant and substantial (>50%) drop in ΔWBA.

For experiments with the lights off, gratings were filtered through a gaussian window, covered a 60° solid angle, and centered at either -45, 0, or 45° elevation. Initially, the experiments with lights off were designed to measure both general visual differences and specific differences below, at, or above the horizon. However, the results were confounded by likely differences in head position and FOV and instead were averaged across the 3 positions to measure general differences. For experiments with lights on, gratings passed through a gaussian window, covered a 90° solid angle, and were centered at the horizon.
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Supplementary Table 1: Relevant parameters from the allometric regressions of abdomen, thorax, head, and eye length in relation to body length per species. Asterisks indicate the level of statistical significance of the corresponding $R^2$ or $b$ based on the small table at the bottom. 84% confidence intervals (C.I.) of $b$ allow for comparisons of the allometric constant between species by considering their overlap. Finding that the C.I.’s do not overlap is statistically equivalent to a Student’s T-test with $\alpha < .05$ (Goldstein and Healy 1995).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Species</th>
<th>$R^2$</th>
<th>$b$</th>
<th>84% C.I. for $b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdomen</td>
<td>D. med</td>
<td>0.64</td>
<td>1.47</td>
<td>0.06, 1.56</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>0.64</td>
<td>1.47</td>
<td>0.06, 1.56</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>0.64</td>
<td>1.47</td>
<td>0.06, 1.56</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>0.64</td>
<td>1.47</td>
<td>0.06, 1.56</td>
</tr>
<tr>
<td>Thorax</td>
<td>D. med</td>
<td>0.50</td>
<td>3.00</td>
<td>0.00, 3.00</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>0.50</td>
<td>3.00</td>
<td>0.00, 3.00</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>0.50</td>
<td>3.00</td>
<td>0.00, 3.00</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>0.50</td>
<td>3.00</td>
<td>0.00, 3.00</td>
</tr>
<tr>
<td>Head</td>
<td>D. med</td>
<td>&lt;.01</td>
<td>0.60</td>
<td>0.00, 0.20</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>&lt;.01</td>
<td>0.60</td>
<td>0.00, 0.20</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>&lt;.01</td>
<td>0.60</td>
<td>0.00, 0.20</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>&lt;.01</td>
<td>0.60</td>
<td>0.00, 0.20</td>
</tr>
<tr>
<td>Eye</td>
<td>D. med</td>
<td>&lt;.01</td>
<td>0.52</td>
<td>0.00, 0.10</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>&lt;.01</td>
<td>0.52</td>
<td>0.00, 0.10</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>&lt;.01</td>
<td>0.52</td>
<td>0.00, 0.10</td>
</tr>
<tr>
<td></td>
<td>D. med</td>
<td>&lt;.01</td>
<td>0.52</td>
<td>0.00, 0.10</td>
</tr>
</tbody>
</table>

Significance Levels:
- $*$: $p < .05$
- $*$*: $p < .01$
- $*$**: $p < .001$
Supplementary Table 2: Relevant parameters from the allometric regressions of eye radius, vertical FOV, horizontal FOV, vertical/horizontal FOV, ommatidial count, IO angle, ommatidial area, and the maximum discernible wavelength, $\lambda_{\text{max}}$. Asterisks signify the same as in Supplementary Table 1.

<table>
<thead>
<tr>
<th>Outcome Trait</th>
<th>Species</th>
<th>$r^2$</th>
<th>$b$</th>
<th>95% C.I. for $b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radius</td>
<td>D. mel</td>
<td>.62**</td>
<td>0.49***</td>
<td>(0.34, 0.65)</td>
</tr>
<tr>
<td></td>
<td>D. maj/maj</td>
<td>.48***</td>
<td>0.11***</td>
<td>(0.21, 0.57)</td>
</tr>
<tr>
<td></td>
<td>D. maj/bag</td>
<td>.29*</td>
<td>0.37**</td>
<td>(0.09, 0.56)</td>
</tr>
<tr>
<td>Vertical FOV</td>
<td>D. mel</td>
<td>.01</td>
<td>.005</td>
<td>(0.11, 0.17)</td>
</tr>
<tr>
<td></td>
<td>D. maj/maj</td>
<td>.09</td>
<td>.14</td>
<td>(0.04, 0.31)</td>
</tr>
<tr>
<td></td>
<td>D. maj/bag</td>
<td>.05</td>
<td>.12</td>
<td>(0.07, 0.32)</td>
</tr>
<tr>
<td>Horizontal FOV</td>
<td>D. mel</td>
<td>&lt;.01</td>
<td>-0.03</td>
<td>(0.06, 0.13)</td>
</tr>
<tr>
<td></td>
<td>D. maj/maj</td>
<td>.01</td>
<td>.04</td>
<td>(0.02, 0.24)</td>
</tr>
<tr>
<td></td>
<td>D. maj/bag</td>
<td>.09</td>
<td>.15</td>
<td>(0.09, 0.35)</td>
</tr>
<tr>
<td>VH FOV ratio</td>
<td>D. mel</td>
<td>.01</td>
<td>.05</td>
<td>(0.03, 0.10)</td>
</tr>
<tr>
<td></td>
<td>D. maj/maj</td>
<td>.09</td>
<td>.09</td>
<td>(0.02, 0.22)</td>
</tr>
<tr>
<td></td>
<td>D. maj/bag</td>
<td>.01</td>
<td>.02</td>
<td>(0.01, 0.09)</td>
</tr>
<tr>
<td>Ommatidial Count</td>
<td>D. mel</td>
<td>.65**</td>
<td>0.48**</td>
<td>(0.34, 0.61)</td>
</tr>
<tr>
<td></td>
<td>D. maj/maj</td>
<td>.56**</td>
<td>0.64**</td>
<td>(0.42, 0.86)</td>
</tr>
<tr>
<td></td>
<td>D. maj/bag</td>
<td>.48**</td>
<td>0.46**</td>
<td>(0.28, 0.64)</td>
</tr>
<tr>
<td>IO angle</td>
<td>D. mel</td>
<td>.44**</td>
<td>-0.32**</td>
<td>(0.05, 0.18)</td>
</tr>
<tr>
<td></td>
<td>D. maj/maj</td>
<td>.05</td>
<td>-0.13</td>
<td>(0.03, 0.08)</td>
</tr>
<tr>
<td></td>
<td>D. maj/bag</td>
<td>&lt;.01</td>
<td>0.04</td>
<td>(0.02, 0.45)</td>
</tr>
<tr>
<td>Ommatidial Area</td>
<td>D. mel</td>
<td>.52**</td>
<td>0.34***</td>
<td>(0.22, 0.52)</td>
</tr>
<tr>
<td></td>
<td>D. maj/maj</td>
<td>.26*</td>
<td>0.57**</td>
<td>(0.20, 0.95)</td>
</tr>
<tr>
<td></td>
<td>D. maj/bag</td>
<td>.15</td>
<td>0.50</td>
<td>(0.07, 1.15)</td>
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<tr>
<td>$\lambda_{\text{max}}$</td>
<td>D. mel</td>
<td>.11</td>
<td>.15</td>
<td>(0.30, 0.01)</td>
</tr>
<tr>
<td></td>
<td>D. maj/maj</td>
<td>.03</td>
<td>.16</td>
<td>(0.19, 0.31)</td>
</tr>
<tr>
<td></td>
<td>D. maj/bag</td>
<td>.05</td>
<td>.44</td>
<td>(0.32, 1.20)</td>
</tr>
</tbody>
</table>
Figures

Figure 1: To compare the visual capacity of desert versus vinegar flies, we used a combination of microscopy, allometry, optical modeling, and virtual reality flight simulation. A. One small (top row) and large (bottom row) subject from each species is displayed in profile view. The letters or black spots within white circles indicate landmarks used in measuring how abdomen (ef), thorax (gh), head (cd), and eye lengths (ab) scale with respect to body length (cdef). B. Likewise, one small and large eye from each species is displayed to give a sense of their eye allometry, with each inset zooming in to resolve the individual ommatidia on the eye surface. C. Contrast sensitivity is partially limited by $D$, the ommatidial diameter, and spatial acuity is inversely limited by $\Delta \phi$, the interommatidial (IO) angle. In a spherical eye, $\Delta \phi = D / R$, and the field of view, $FOV$, is equal to the sum of IO angles subtended. D. A computer generates sinusoidal moving gratings projected at 360 Hz onto 5 sides of the perspex cube using 4 angled mirrors. E. In the flight arena, a shadow of both wings is cast by an IR LED and their wingbeats are recorded independently by two photodiodes at 1 kHz. F. The difference in left and right wingbeat amplitudes ($\Delta WBA$) is proportional to yaw torque and implies their perception of motion via the optomotor response. The $\Delta WBA$ time series collected from one subject in response to 11 different grating conditions are plotted, with colors indicating grating conditions: a grating of .0 contrast showing no motion (yellow) and 5 contrasts (.09, .28, .47, .66, and .85) moving towards the left (warm colors) and right (cool colors).
Figure 2: We propose a novel method for measuring the eye radius of spherical compound eyes. A. Using the digital recording microscope, multiple images of the eye are taken from one angle at fixed intervals of focus depth. B. A focus stack is generated by taking each pixel of greatest focus (approximated by local contrast as in D.) from the stack of images. C. The curvature of the eye can be corrected for once you know the eye radius and center. D., E. The 3D eye surface is approximated by taking measurements of local contrast for each image by finding the height that maximizes local contrast for each pixel. F. The eye radius and center are derived by fitting a sphere to the 3D eye surface. FOV was calculated as the area of a best fitting ellipse to the outline of the eye in spherical coordinates (C.) and its vertical and horizontal components as the major and minor diameters.
Figure 3: To measure general morphological differences between *D. mel* (grey), *D. moj moj* (red), and *D. moj baja* (blue), we compared abdomen (A.), thorax (B.), head (C.), and eye length (D.) and their relation to body length (x-axes). General differences are shown in box plots corresponding to each axis while allometric differences are shown in the scatter plots. Each box plot shows the sample median (black tick), IQR (upper and lower bounds of the box), range (whiskers), and outliers (fliers). Brackets point to statistically significant differences based on pairwise Student’s T tests using Šidák-Holm corrected p-values signified by asterisks: * p < .01, ** p < .0001. Each scatter plot shows the allometric regression mean (solid lines) and 84% confidence bands (translucent segments).
Figure 4: Left column: To measure visual field differences between *D. mel* (grey), *D. moj moj* (red), and *D. moj baja* (blue), we compared eye radius (A.) and vertical and horizontal FOV (B. and C.) and their relation to eye surface area (x-axes). Middle column: for optical differences, we compared ommatidial count (D.), IO angle (E.) and ommatidial area (F.) and also their relation to eye surface area (x-axes). Box plots, significance brackets, scatter plots, and regression plots are the same as in Figure 3. G. A dorsal view of the three genotypes shows that the eyes in desert flies (*D. moj moj* and *D. moj baja*) recede further into the thorax, extending their visual field horizontally more than vinegar flies (*D. mel*).
Figure 5: Optomotor response of *D. mel* (grey) and *D. moj moj* (red) to 10 different contrasts (x-axes) in three different lighting conditions: room lights on with a neutral density (ND) filter applied to the projector (column 1) and without (column 2) or room lights off without an ND filter applied (column 3). In each heatmap, the y-axis is time and the color at each time point indicates the ΔWBA, normalized to the maximum per species (blue signifies ΔWBA < 0, indicating counter steering). Mean responses are plotted in the margins: interspecific comparisons per lighting condition in the bottom row and intraspecific comparisons across lighting conditions in the right column. Error bars indicate the standard error of the mean using bootstrapping to account for repeated measures. Plus symbols below the x-axis of each marginal plot indicate that the mean is significantly greater than 0 based on the lower bound of their bootstrapped 99% CI. With Michelson contrast, contrast sensitivity is defined as 1 / $C_{\text{min}}$, where $C_{\text{min}}$ is the lowest contrast they respond to significantly. For each row of plus symbols, the fully saturated sign indicates $C_{\text{min}}$. 
Figure 6: Optomotor response of *D. melanogaster* and *D. mojavensis mojavensis* as outlined in Figure 5 but to 10 different spatial frequencies measured in cycles per degree (CPD). Spatial acuity is defined as the maximum detectable spatial frequency as indicated by the fully saturated plus symbols.
Figure 7: Optomotor response of *D. melanogaster* and *D. mojavensis mojavensis* as outlined in Figure 5 but to 10 different temporal frequencies measured in cycles per second (Hz). Temporal acuity is defined as the maximum temporal acuity preceding a substantial drop in response (>50%), as indicated by the fully saturated plus symbols.
CHAPTER V
CONCLUSIONS AND FUTURE DIRECTIONS
The previous chapters presented a method for automatically characterizing compound eyes (chapter 2) and demonstrated the developmental plasticity of eye morphology, neural summation, and optomotor behavior within one fruit fly species (*Drosophila melanogaster*; chapter 3) and how modifications in eye development and neural summation strategies between two fruit fly species (*D. melanogaster* and *D. mojavensis*; chapter 4) can enable successful optomotor behavior across very different visual environments and ecologies.

In chapter 2, we proposed a method and computer program for automating the detection of ommatidia in images and 3D datasets. By leveraging the spatially periodic arrangement of ommatidia, the two programs automatically detect ommatidia location and size to measure how parameters like spatial acuity and contrast sensitivity vary across the visual field. This project has branched into multiple parallel collaborations applying the ommatidia detecting algorithm (ODA) to large collections of images of fruit flies (my own ongoing work), mosquitos (with Dr. Matthew J. DeGennaro’s lab at FIU), moths (with Dr. Akito Kawahara’s lab at UF), and butterflies (with Dr. Daniel Speiser’s lab at USC), which are producing promising results.

In chapter 3, we demonstrated that smaller fruit flies have smaller eyes composed of fewer and smaller ommatidia, which sacrifice optical sensitivity more than spatial resolution. However, due to the likely impact of neural summation, we also tested the spatial and temporal acuity and contrast sensitivity of their optomotor response. When flying in virtual reality, small and large conspecifics actually demonstrated equivalent contrast sensitivity and comparable spatial acuity. We found that small flies achieve this
through temporal summation, sacrificing the detection of high temporal frequencies in order to maintain comparable levels of contrast sensitivity.

Finally, in chapter 4 we found that desert fly (*D. mojavensis*) eyes provide higher spatial acuity, lower contrast sensitivity, and wider field of view than in vinegar flies, befitting the characteristically bright, barren, and horizontal desert panorama. We applied the ODA from chapter 2 to characterize the optical tradeoffs of the two species. Similar to the vinegar fly, small desert fly conspecifics also sacrifice contrast sensitivity but appear to maintain spatial acuity based on eye morphology. Nonetheless, when tested under various light levels, the two species used different neural summation strategies, with desert flies spatially and temporally summatating to a greater extent but only after a large change in ambient brightness.

**Future Directions**

**Inverse Temperature Rule**

The use of temporal summation in response to reduced light absorption, as we found in chapters 3 and 4, also occurs during light adaptation of individual fly photoreceptors, which optimizes the signal-to-noise ratio as a function of ambient light level (Juusola & Hardie, 2001). However, dark adaptation does not induce temporal summation to the extent that we measured in the small flies of chapter 3. Instead, these responses are closer to the cumulative effect of reduced photoreceptor activity over the first five days post-eclosion caused by dark rearing. The impulse response of photoreceptors of flies reared in the dark versus normal light are about 20% slower and 26% smaller (Wolfram & Juusola, 2004). Dark rearing also reduces the volume of the photoreceptors and three of the four visual neuropils involved in wide-field motion detection (lamina, medulla, and
lobula plate; Barth et al., 1997). So, we speculate that the effect of smaller ommatidia within a species is similar to dark rearing because it reduces the light available for phototransduction, having a cumulative effect on photoreceptor and visual development. To address this, we are currently exploring a different factor known to affect insect organ allometry: thermal plasticity. All animals, including insects, follow a general tendency called the inverse temperature rule by which animals exposed to higher temperatures tend to develop into smaller adults with smaller organs (Davidowitz & Nijhout, 2004). However, our measurements suggest that, as opposed to general body size and the size of other organs like the wings and legs (Davidowitz & Nijhout, 2004; Ghosh et al., 2013), temperature does not affect eye size in fruit flies (at least between 16.5℃ and 26.5℃). Instead it alters the composition of the eye, with colder temperatures resulting in eyes with more but smaller ommatidia (Figure 1). More work is needed to understand the effect on the optomotor response and neural summation, but the plasticity of eye development to temperature allows us to measure the separate effects of ommatidia count and ommatidia size on the visual performance of the optomotor response within the same species. Moreover, any thermal plasticity of the optomotor response has ecological implications given seasonal and anthropogenic changes in ambient temperature.

**Periphery and Optical Flow**

The pattern of visual motion perceived during forward movement includes fast peripheral motion that is useful in determining information about the structure of the environment, like relative flight speed and orientation. In many flying insects, these high speeds are resolved optically through greater interommatidial angles and larger ommatidial apertures in lateral regions of the eye to improve sensitivity. But fruit fly eye optics are nearly
homogeneous and the temporal summation we found in their central vision would cause substantial motion blur in the periphery. Therefore, we expect small flies to use different neural strategies in their central and peripheral visual field, to maximize useful contrast in both regions. This is similar to the terrain hypothesis discussed in chapter 4 but must be faced by all forward flying insects.

In an ongoing project, we are testing this hypothesis by measuring the different neural strategies used in central versus peripheral vision and by modelling the optical sacrifices faced by small flies (Figure 2). The preliminary results corroborate the finding in chapter 3 that smaller eyes, which have lower optical sensitivity, temporally summate in their central vision (Figure 2 A, top row). However, when motion was displayed in their periphery, small and large flies respond nearly equally, though with a small loss in contrast sensitivity (~10%). Surprisingly, small flies, which have clear optical disadvantages, show minimal peripheral visual losses at the behavioral level. They achieve this by accepting a certain optical phenomenon called spatial aliasing, in which certain high spatial frequencies are undersampled, resulting in the perception of motion opposing the true direction and optomotor countersteering (Figure 2 A, bottom right, red arrow).

Using a model of elementary motion detection, we find that these behavioral results are very likely the effect of a reduced acceptance angle of the individual photoreceptors (Figure 2 B). EMD simulations reveal how a narrower acceptance angle results in a general increase in contrast sensitivity (Figure 2 B, middle) by increasing the contrast of low spatial frequencies but introducing spatial aliasing (Figure 2 B, right). Ongoing work will measure these acceptance angles directly by using intracellular electrophysiology in
photoreceptors of the central and peripheral visual field of small versus large conspecifics and will compare microCT scans of small and large conspecifics to understand the underlying optical cause. This work should elucidate the cellular and structural mechanisms underlying the visual adaptation to optic flow.

**Monitoring Light-Related Circadian Activity**

At the end of chapter 3, we speculate that small flies cope with their reduced temporal acuity by requiring more ambient light in general. On a standard daylight cycle, this means they should start their day later and end it earlier. However, conventional lab husbandry uses a binary 12 hours on, 12 hours off boxcar light cycle, obfuscating any plasticity in light-induced behavior. We are testing this by using incubators modified to provide light simulating a gradual sunrise and sunset. The incubators are equipped with 32 parallel activity monitors capturing automatic measurements of movement in real time. More work is needed to understand the effect of body and eye size on their activity patterns, but preliminary work shows that the gradual light indeed changes their activity. In particular, both conditions show crepuscular activity but flies in the boxcar condition are most active during sunrise while those from the gradual condition are most active during sunset (Figure 3). If there is a difference in activity schedules, this paradigm should allow us to measure it.

**Conclusion**

This manuscript attempted to make progress in understanding the role of fruit fly visual plasticity (morphological or otherwise) in the development and evolution of vision. First, it offered a tool to accelerate large-scale research into compound eye morphology, which is proving successful on the eyes of several insect orders. Then, it demonstrated the
developmental plasticity of eye morphology and neural summation in fruit flies, finding an interesting interplay between the two systems. It also elucidated the role of visual plasticity and neural summation in the evolution of vision by comparing vinegar and desert flies. Finally, future and ongoing work is diving into the mechanisms underlying this visual plasticity by measuring the effect of early temperature, exploring regional differences across the visual field, and detecting light-induced circadian activity.

References


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Figure 1: Vinegar fly (*Drosophila melanogaster*) eye size (x-axis), ommatidia count (y-axis, top), ommatidia diameter (y-axis, bottom) and their allometries under 16.5°C (blue) and 26.5°C (red). Asterisks indicate that means are significantly different using an independent samples T-test: * P < .05, ** P < .01.
Figure 2: A. Optomotor responses to varying contrasts (left column), temporal frequencies (middle), and spatial frequencies (right) presented in the central (top row) and peripheral visual field (bottom row). The gray value in the background of each plot corresponds to the relative performance of small versus large fly performance. B. EMD simulation results showing how a narrower acceptance angle (green versus grey) results in a general increase in contrast sensitivity (middle) because of the increased contrast of low spatial frequencies combined with spatial aliasing to high frequencies (right). The units on the y-axes of the middle and right plots are arbitrary.
Figure 3: Mean ± S.E.M. activity for boxcar (left) and gradual (right) light cycles over 6 days post eclosion. The background gray values indicate the relative ambient brightness of each condition. Note that the flies are most active during sunrise in the boxcar condition while they are most active during sunset in the gradual condition.
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