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Functional Regionalization in the Fly Eye as an Adaptation to Habitat Structure

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

FUNCTIONAL REGIONALIZATION IN THE FLY EYE AS AN ADAPTATION TO HABITAT STRUCTURE

A dissertation submitted in partial fulfillment of
the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
BIOLOGY
by
Carlos Alberto Ruiz

2021

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

This dissertation, written by Carlos Alberto Ruiz, and entitled Functional Regionalization in the Fly Eye as an Adaptation to Habitat Structure, 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.

Robert Lickliter

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Jamie Theobald, Major Professor

Date of Defense: March 22, 2021

The dissertation of Carlos Alberto Ruiz is approved.

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

Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2021

DEDICATION

To the memory of my mother and the cheering presence of my wife.

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On the personal side, I would like to thank my wife Cleopatra Pimienta who has held my hand during all this time and has loved me, as I love her; and Dr. Phil Stoddard for standing by my side during the darkest of times, his words always gave me hope and strength and I will never forget them. Along with him, Dr. Scott Fingerhut who taught my wife and I how to hold our ground in our search for justice; my advisor Dr. Jamie Theobald and his wife Dr. Melissa Coates, and Dr. Jeffrey Wells, who were always there to listen to me, provide counsel, and helped me carrying the weight of my burden.

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ABSTRACT OF THE DISSERTATION

FUNCTIONAL REGIONALIZATION IN THE FLY EYE AS AN ADAPTATION TO HABITAT STRUCTURE

by

Carlos Alberto Ruiz

Florida International University, 2021

Miami, Florida

Professor Jamie Theobald, Major Professor

With over 150,000 described species, flies constitute one of the most species-rich groups of animals on earth, and have managed to colonize almost every corner of it. Part of their success comes from their amazing flying skills, which are strongly tied to their visual capabilities. To navigate quickly and accurately through their habitats, they must be able to process the inordinate amounts of visual information necessary to sort obstacles, avoid predators and remain on course. Surprisingly, despite their tiny brains, flies have no problem in processing all of that information to generate correcting maneuvers in just about 30 milliseconds. To this end, the fly eye is extremely specialized for the perception of motion following the anisotropies of the environment, but that is only part of the story. In my dissertation I investigated whether this regional specialization of the eye extends to other characteristics of the stimulus that provide structural information about the habitat. I focused on three main questions: (1) do flies regionalize the perception of parallax across regions of the eye? (2) Is there regionalization in the perception of the spatial layout of visual elements during flight? (3) How is this regional processing modified in species adapted to different habitats? Using a virtual reality setup to measure flight behavior with very high precision, I tricked the flies into responding to a sudden and unintended change in position, signaled by strong visual sideslip of their visual environment. I found that during these disturbances, (1) flies respond to motion parallax only if it is below them, not above; (2) the same pattern of response applies to the layout of visual elements moving during the disturbance; (3) while the basic mechanisms are present across species adapted to different habitats, responses are modulated differently, signaling evolutionary novelties in specialized natural histories. My findings advance our understanding of the ways in which processing of visual information in flying insects is optimized for speed and accuracy of responses, when neural resources are limited. It also offers insights into how highly conserved behaviors are modulated in species adapted to completely different environments.

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PREFACE

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CHAPTER II

Ruiz, C. & Theobald, J. C. 2020. *Ventral motion parallax enhances fruit fly steering to visual sideslip*. Biology Letters 16 (5): 20200046. https://doi.org/10.1098/rsbl.2020.0046

CHAPTER III

Ruiz, C. & Theobald, J. C. 2021. *Stabilizing responses to sideslip disturbances in Drosophila melanogaster are modulated by the density of moving elements on the ground*. Biology Letters 17 (3): 202000748. https://doi.org/10.1098/rsbl.2020.0748

CHAPTER I

INTRODUCTION

The order Diptera contains more than 150,000 described species of flies (Courtney et al., 2009; Irwin et al., 2003), making it one of the four most specious groups of insects. Diptera surpasses any other order of insects in ecological diversity (Grimaldi and Engel, 2005). Having colonized all continents and assumed a multitude of lifestyles, flies are incredibly diverse in morphology and natural histories (Courtney et al., 2009).

One of the key factors propelling their success as a group is their impressive aerial maneuverability. Flies are able to traverse environments of varied structure at high speed, avoid obstacles and correct course deviations; either while chasing a mate, hunting their prey, or avoiding becoming prey themselves. Their maneuverability is, in part, a result of extreme modifications associated with their motor system. In contrast to any other group of insects, flies modified their second pair of wings into an additional set of sensors, the halteres, which can detect changes in direction caused by small rotations during flight (Pringle, 1948). The concomitant loss of the additional lift provided by the second pair of wings, was accompanied by the strengthening of the middle section of the thorax, which powers flight (Grimaldi and Engel, 2005). This highly modified external morphology, added to the presence of asynchronous indirect flight muscles that focus solely on thoracic deformation (Dickinson and Tu, 1997; Nachtigall and Wilson, 1967), seem to have played a critical role in the evolution of the high wingbeat frequencies necessary for fast and precise flight (Dudley, 2002). While being able to fly fast and detect rotational disturbances is critical for the long migrations involved in exploring new habitats, the accurate navigation of cluttered environments demands a detailed evaluation of translational motion, to control flight speed and prevent collisions. Since mechanosensory systems are only sensitive to acceleration, the evaluation of flight speed during translation is mostly a visual task (Budick et al., 2007). Although visual input is used to perceive and correct both rotational and translational disturbances, flies' eyes seem to be more sensitive to positional than to directional deviations, as shown by the much stronger stabilizing responses elicited by this type of disturbances (Tammero et al., 2004). However, opposed to mechanosensory systems, which directly perceive the magnitude of an acceleration, the visual system does not directly perceive motion. The perception of motion and subsequent evaluation of velocity, are indirect results of the intense processing of the only type of information the visual system perceives, which is spatio-temporal patterns of contrasts detected by photoreceptors across the surface of the eye (Reichardt, 1987).

During flight, flies experience a predictable pattern of flow of visual elements across their eyes that is determined by relative prevalence and direction of rotational and translational components (Borst et al., 2010). During changes in direction, such as yaw rotations, flies experience a characteristic pattern of retinal slip affecting all of the elements in their field of view in the same way, regardless of their distance (Koenderink, 1986). To correct a rotational disturbance, a fly only needs to move in the same direction suggested by this optic flow until all the elements are back to their original positions. This simple mechanism however, does not work with translation. During forward flight for example, the optic flow is composed of elements moving front to back, whose visual speed increases as they move from the centre of expansion in front toward the centre of contraction behind, reaching their peak velocity when they pass to the side of the fly (Koenderink, 1986). While this pattern of optic flow is common to all sorts of translations, the actual retinal speeds of individual elements in it is determined by their distances to the fly. Images of distant objects move through the retina more slowly than images of near ones, just like distant mountains move much more slowly than near trees as you drive on a highway. Since the relative velocities of the individual elements are determined by the distance between them and the travelling fly, their relative motion conveys information about the 3D structure of the environment. Relative motion, or in other words, motion parallax is one of the most important depth cues for organisms that lack effective stereo vision, such as flies (Sobel, 1990; Srinivasan et al., 1990).

Besides the spatial information of the environment obtained during translation, the actual layout of elements in the surroundings, along with their texture, color, reflectance and others, can affect the spatio-temporal patterns of contrasts perceived by the fly eye, which are the basic input used by the visuo-motor system for navigation. The actual placement of contrasting elements in the field of view, or spatial distribution of contrasts, is strongly affected by the presence and level of heterogeneity of structured vegetation (Prokopy and Owens, 1983). Similarly, the lack of a canopy for example, allows more light to reach the ground creating sharper shadows among strongly illuminated patches of soil, dramatically increasing the range of contrasts present in the visual scene (Mante et al., 2005). Regardless of its immense advantages, visual input must be rapidly and thoroughly processed to generate timely motor responses, otherwise it is useless. The demand for fast processing of visual input poses a serious challenge for flies, due to the large amount of information contained in it, and the limited neural resources available to evaluate it (Chittka and Niven, 2009). Flies seem to have addressed this problem by focusing the processing of specific types of motion vectors in regions of the eye where they are usually prevalent. This led to functional differentiation between the dorsal and ventral regions of the eye, according to the general structure of the visual world of the fly, making the lower portion of the eye more responsive to translation than the upper (Cabrera and Theobald, 2013; Krapp and Hengstenberg, 1996). However, optic flow can also provide information about habitat structure that is relevant during navigation, as demonstrated by the general effect of parallax in the modulation of stabilizing responses in the vinegar fly (Cabrera and Theobald, 2013). It is likely then that the processing of stimulus parameters providing information about the layout of elements in the environment, shows at least some level of regionalization helping to reduce the processing load on the visual system. So far, the neural elements involved in the perception of parallax have not been characterized in fruit flies yet, but in blowflies these neurons are known to extend to the ventral region of the eye (Longden et al., 2017). Since parallax is exclusive of translation, the enhanced sensitivity to it on the ventral region of the eye suggests the possibility of regionalization in the processing of similar stimulus parameters in other flies.

In this thesis, I sought to find whether flies do indeed have independent regional sensitivities for stimulus parameters containing information about the physical structure of the environment, and investigated how those sensitivities are tuned in species adapted to different habitats. To this end, fruit flies of the family Drosophilidae offer an ideal group of model organisms. With over 3500 species, the family is considered one of the most ecologically diverse groups of dipterans (Grimaldi and Engel, 2005). The family is thought to have gone through several events of adaptive radiation, leading to their widespread distribution and divergence of lifestyles (O’Grady and DeSalle, 2018). Thanks to their diverse ecology, it is relatively easy to find species of fruit flies with contrasting natural histories, yet closely related phylogenetically. In order to study their flight behavior, I used a virtual reality setup where flies could respond to visual stimuli while tethered. The study of insect flight under tethered conditions provides high-precision readings of wing-beating patterns, at a temporal resolution that allows the detection of changes in steering behavior every millisecond, as the subjects respond to visual stimuli.

In Chapter II, I evaluate the effect of 3D structure of the environment on the stabilizing responses of *D. melanogaster*, across the dorsal and ventral regions of the eye. As a result of the relevance of motion parallax in the perception of depth, the differential responsiveness to it across eye regions could be related to the general prevalence of motion vectors signaling translation on the ground, and may not signal adaptations to a particular habitat. To ensure that the responses found are caused exclusively by the perception of depth during translation, I compare them with responses to relative motion during rotation, a condition that does not exist in nature. The findings presented in Chapter II demonstrate the existence of regional processing of visual cues associated with habitat structure in the vinegar fly. My results further our understanding of how small flies optimize the processing of complex optic flow. Chapter II has been published in Biology Letters.

In Chapter III, I proceed to evaluate the effect of the spatial distribution of surrounding contrasts on stabilizing responses in *D. melanogaster*. During sudden changes in position, the density of elements moving in the optic flow contains information about the general structure of the environment. The fine tuning of motor responses in *D. melanogaster* within a range of element densities on the ground, suggests that visually-driven steering responses in flies could be tuned to the visual structure of particular habitats. My findings further our understanding of the role that the physical structure of the environment has on flight behavior in the vinegar fly. Chapter III has been published in Biology Letters.

In Chapter IV, I present a comparative study of the responses to variations in the 2D layout of elements in motion, across species of fruit flies from different habitats. I compare responses between two fruit fly species adapted to habitats with dense vegetation, and one adapted to a desert environment. The findings that I present here shed light on how the modification of patterns of response to structural parameters of the environment, could act as adaptive traits facilitating survival in completely different environments.

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CHAPTER II

VENTRAL MOTION PARALLAX ENHANCES FRUIT FLY STEERING TO VISUAL SIDESLIP

Carlos Ruiz and Jamie C. Theobald

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Abstract

Flies and other insects use incoherent motion (parallax) to the front and sides to measure distances and identify obstacles during translation. Although additional depth information could be drawn from below, there is no experimental proof that they use it. The finding that blowflies encode motion disparities in their ventral visual fields suggests this may be an important region for depth information. We used a virtual flight arena to measure fruit fly responses to optic flow. The stimuli appeared below (n=51) or above the fly (n=44), at different speeds, with or without parallax cues. Dorsal parallax does not affect responses, and similar motion disparities in rotation have no effect anywhere in the visual field. But responses to strong ventral sideslip (206 deg/s) change drastically depending on the presence or absence of parallax. Ventral parallax could help resolve ambiguities in cluttered motion fields, and enhance corrective responses to nearby objects.

Keywords

parallax, insect vision, optomotor response, optic flow, *Drosophila melanogaster*

Introduction

Flies execute extremely fast and precise aerial manoeuvres, requiring robust corrective responses to handle deviations from course. They use coherent motion from optic flow fields to countersteer against changes in direction and position during flight [1, 2]. For this purpose, background motion is decomposed into translational and rotational components that are processed independently by large field neurons in the lobula plate in flies [1-6], and arthropods in general [7].

Responding to translational self-motion requires nearby visual features, because image speed on the retina varies inversely with object distance [8]. Rotational image speeds, in contrast, are unaffected by distance. Some flies take advantage of this difference by increasing their sensitivity to translation in the frontolateral and subequatorial regions of their eyes, where perceived objects are usually closer during natural flight, while displacing the perception of rotation to the dorsal region [4]. *Drosophila*, for example, can use celestial cues above for evaluating changes in direction (reviewed by Warren *et al.* [9]), while positional tasks such as groundspeed control or responses to sudden changes in position are mostly based on optic flow below and near the horizon [10-12].

Translational optic flow additionally provides cues about the three-dimensional structure of the surroundings, which manifests as image speed being inversely proportional to object distances [8, 13, 14]. Flies can process motion patterns from the frontolateral regions of the visual field and use them to gauge distances [15,16], and separate objects from background [17-19], both critical tasks for navigating through cluttered environments. But motion depth cues are present outside of the frontal or lateral visual field. When flying low over patchy vegetation, for example, a wealth of information about the spatial distribution of features is available right underneath. Bees use this for altitude control [20-22], but flies, for some reason, do not [10, 26]. Whether this is because they fail to integrate relative motion beneath, or shift attention to frontal areas during forward flight [27], remains unknown.

The recent finding of a neuron (VT1) in the blowfly *Calliphora vicina*, able to encode motion parallax in the forward and sideslip directions below the horizon [28], provides a partial answer to this question. It demonstrates that at least some groups of flies encode parallax in ventral optic flow, and suggests that this trait could be adaptive to flies traversing habitats with obstacles [29] or foraging for resources on the ground. This could be the case in *D. melanogaster,* a slow flier that searches for fallen fruit.

Despite the abundance of derived traits associated with the lobula plate tangential cells (LPT) across groups of flies [30], horizontal system cells (HS) responsible for assessing yaw-rotation are relatively conserved between blowflies and fruit flies [31]. It is therefore possible that they also share the ability to perceive and encode incoherent motion below during flight. We set out to test whether parallax affects the optomotor response of fruit flies during visual perturbations in the ventral or dorsal region of the flow field. We used a virtual flight arena to display perturbations, with and without depth cues, and measure optomotor responses of tethered fruit flies.

Methods

Experimental subjects

We tested *Drosophila melanogaster* females within 3-5 days of eclosion, reared in the laboratory under a 12 L: 12 D cycle, kept at 21°C, and fed standard medium. Flies were cold anesthetized, then glued to a fine tungsten rod by the mesonotum. They recovered in the dark for at least 30 minutes while holding a small piece of paper with their legs, preventing them from flapping their wings. We then removed the paper when suspending each fly in the centre of the arena (figure 1A). Each fly was tested only once in an experiment.

Virtual flight arena

We projected visual stimuli onto the lower and upper surfaces of a 200 mm Perspex cube (figure 1B). Perspective-corrected stimuli displayed in a 90° diameter disk. Experiments took place in a dark room to increase contrast, and the sides of the cube prevented flies from getting light from any other direction. Further details of the arena are described in Cabrera & Theobald [32].

Visual stimuli

Each experiment consisted of open loop presentations of dot-fields moving either rightward (clockwise in rotation), or leftward (counterclockwise), projected to the ventral or dorsal visual region of the fly (see the electronic supplementary materials for details (video 1)). Dot-field motion was either rotational or sideslip, at 1 of 4 different angular speeds, and with or without parallax depth cues (figure 1C). We emulated depth cues by adding relative motion to a randomly distributed group of dots moving in the same direction, suggesting increased distance [12] (figure 1D). This ensured the number of dots was constant (113 dots/steradian), and allowed us to add differential speeds to rotational flow fields, which intrinsically have no such feature (see the electronic supplementary materials for details on the stimuli used (videos 2-5)). The trials were presented in random order, and interspersed by segments of closed-loop bar fixation to standardize the behavioral state at the beginning of each test [33,34].

Steering responses

Tethered flies were illuminated from above with an infrared light, while photodiodes below measured the shadow produced by each wing beat. Since flies steer by changing the relative amplitudes of left and right wing beats [35], attempts to turn produce a differential voltage by the sensor pair [36,37], which is reported as the voltage difference in wing beat amplitude (ΔWBA). Responses collected include roll and yaw attempts performed by the fly, as they both result from the same flight mechanics, and are indistinguishable using a wing beat analyzer [2].

Results

Flies responded to dorsal and ventral stimuli by steering in the direction of the flow, and increasing amplitude with flow speed. Coherent sideways flow with angular speeds up to 138 deg/s elicited responses of similar amplitude when presented dorsally and ventrally (figure 2A and B, blue lines). However, high speeds of sideways flow that suggested stronger disturbances with coherent motion (206 deg/s), produced significantly weaker ventral responses (t=-2.365, p=0.023).

Due to the local optimisation of the dorsal region of the eye for evaluating rotation [4], if flies are sensitive to parallax, they might respond to it only when it is present in ventral disturbances. Since motion parallax is exclusive to translation, we expected flies to be unresponsive to it when was added to rotation. For optic flow including relative motion (simulating parallax), response to strong ventral sideslip disturbances increased significantly (t=-2.313, p=0.023) (figure 2D, green trace). This was similar in amplitude to the response to a dorsal stimulus, either in the absence or presence of depth cues (t=-0.001, p=0.999 and t=-0.227, p=0.821, respectively) (figure 2C). Relative motion had no effect on steering response when added to dorsal sideslip (figure 2A green) or rotation (figures 2E and F). This occurs even at high-speed translational disturbances presented dorsally (t=0.23, p=0.819) (figure 2A green), or rotational in both regions of the visual field (figure 2G and H, green).

Discussion

Response to dorsal and ventral sideslip disturbances without depth cues

In contrast to hawkmoths *Manduca sexta*, that maintain flight control even with the ventral region of their eyes covered [38], steering responses to positional changes in flies may be strongly based on flow fields below the horizon, as demonstrated in blowflies [4]. In fact, flies respond weakly to translational cues present only in the upper visual hemisphere [12]. However with the narrower dorsal and ventral visual fields shown here, low-speed disturbances containing only coherent motion elicited similar steering responses in both of these regions. Only high speed disturbances caused a strong difference in the weakening of ventral perturbations without parallax.

Similar steering response shifts are seen during forward flow. Flies shift attention to anterior regions of the ventral flow-field as forward flow speed increases [27]. This may alleviate motion blur [25] by focusing attention on areas with slower optic flow, potentially reducing responsiveness to perturbations below. Fast optic flow can also induce spatial summation, forcing the fly to spatially pool information in lateral regions. This increases the ability to respond to fast stimuli at the cost of spatial resolution [39]. It is unknown if sideways perturbations can trigger such neural strategies.

Response to wide-field incoherent motion

As expected, relative motion cues affected steering responses only in sideslip, and not yaw rotation. Since rotational and translational components of motion are processed separately [1, 3, 4], encoding relative motion may be a property of large-field neurons, responsible for the translational components of self-motion only. Further, the similarity in the responses to translating dot-fields with and without relative motion in the dorsal region of the eye, suggests the presence of a less specialized system for the perception of translation there, in contrast with regions near the horizon where translational cues are more relevant [12]. While incoherent motion is known to be informative in frontolateral regions [15, 16], our results extend that range to the ventral region, showing that fruit flies perceive, encode, and use depth cues below them. Due to the conservation of traits inherent to the perception of rotation between blowflies and fruit flies [31], we believe elements involved in the integration of depth cues in the ventral flow field in *D. melanogaster* may be homologous to those found by Longden *et al.* [28] in *C. vicina*. Due to strong selective pressures acting on LPT cells [30], the ability to perceive ventral parallax in *D. melanogaster* is a selective trait, with a variety of possibly adaptive roles.

Height control

Although the depth cues from the ventral optic flow could be used for height control when flying over structured environments (bees are an example [20-24]), fruit flies seem to control their height during flight using information from frontolateral areas of the visual field [10, 26], while referring to ventral optic flow in order to control groundspeed [10,40]. Because the effect of relative motion was noticeable only during strong sideslip, ventral parallax is probably not involved in altitude control, but further experiments with different levels of parallax would be required to rule it out completely.

Dealing with ambiguity

Different types of self-motion can generate identical flow fields when perceived by small regions of the eye [4, 41], and partial stimulation of wide-field neurons could therefore be ambiguous. In our experiment for example, moving dots in the small ventral visual field could be perceived by the fly as either sideslip or a roll. However, the presence of parallax in the ventral optic flow could confirm that an otherwise ambiguous stimulus results from translation, because incoherent motion is absent from rotation. However, this is complicated because VS neurons sensitive to roll branch out laterally instead of ventrally on the eye in blowflies [42-45], suggesting that lateral motion on a small region of the ventral field is possibly perceived only as translational, which could also apply to *Drosophila*.

Navigating complex environments

Our results suggest the presence of relative motion ventrally, even in a narrow cone of vision, is enough to prevent attention from shifting forward, and keep the fly responsive to potential risks below during strong perturbations. The sense of nearness produced by motion parallax induces stronger corrective responses to sideslip disturbances in fruit flies [32]. The fact that fruit flies share the ability to encode parallax information from ventral flow fields with blow flies is not that surprising. The saprophagous nature of both flies forces them to move around in search of ephemeral resources that can be far apart and usually at ground level. While *C. vicina* is a fast flier that moves across patches of differently structured vegetation [29], *D. melanogaster* can forage longer distances and even migrate if necessary in search of resources [46]. With such a natural history, both species can certainly benefit from being aware of the dangers below when traversing unknown structured environments.

We have demonstrated that fruit flies respond to the presence of parallax during strong sideways disturbances in their ventral optic flow. The robustness of this response suggests that it is an adaptive trait, but its full significance is unresolved.

Data accessibility

Data are available from the Dryad Digital Repository:

https://doi.org/10.5061/dryad.2jm63xskb.

Electronic supplementary material is available online at:

https://doi.org/10.6084/m9.figshare.c.4966274.

Authors' contributions

C.R. and J.C.T. designed the experiment and wrote the manuscript. C.R. collected and analysed the data. Both authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests

We declare we have no competing interests.

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Figure Captions

Figure 1. (A) Rear view of the projection arena showing the placement of the fly. The IR led above illuminates the wings in motion casting a shadow on the sensor below the fly. (B) The stimulus projects onto the ventral or dorsal faces of a Perspex cube using mirrors. (C) Steering attempts are inferred from the difference in the size of shadows of the left and right wings captured by the dual sensor. Mean steering responses of the flies tested can be visualized as time series (solid lines), along with the standard error of the mean SEM (shading). (D) Motion parallax in the stimuli is simulated by adding relative motion to the dot-field elements.

Figure 2. (A), (B) Mean response of *Drosophila melanogaster* to unintended sideslip containing only coherent motion (blue), and with relative motion suggesting the presence of parallax (green). The stimuli were presented at 4 speeds and two directions on the dorsal and ventral regions of the visual field. Responses were averaged between 0.2-0.4s after stimulus onset. (C), (D) Time series showing the first 0.4s of the mean response to the highest speed sideslip (206 deg/s) with and without depth, in the dorsal and ventral visual fields. (E), (F) Mean responses to rotational stimuli with and without relative motion, presented in the dorsal and ventral visual fields. (G), (H) Time series showing the response of the flies to both types of motion at high speed in their dorsal and ventral visual fields. Solid traces represent mean responses obtained from *n* flies, shading represents SEMs.

Figures

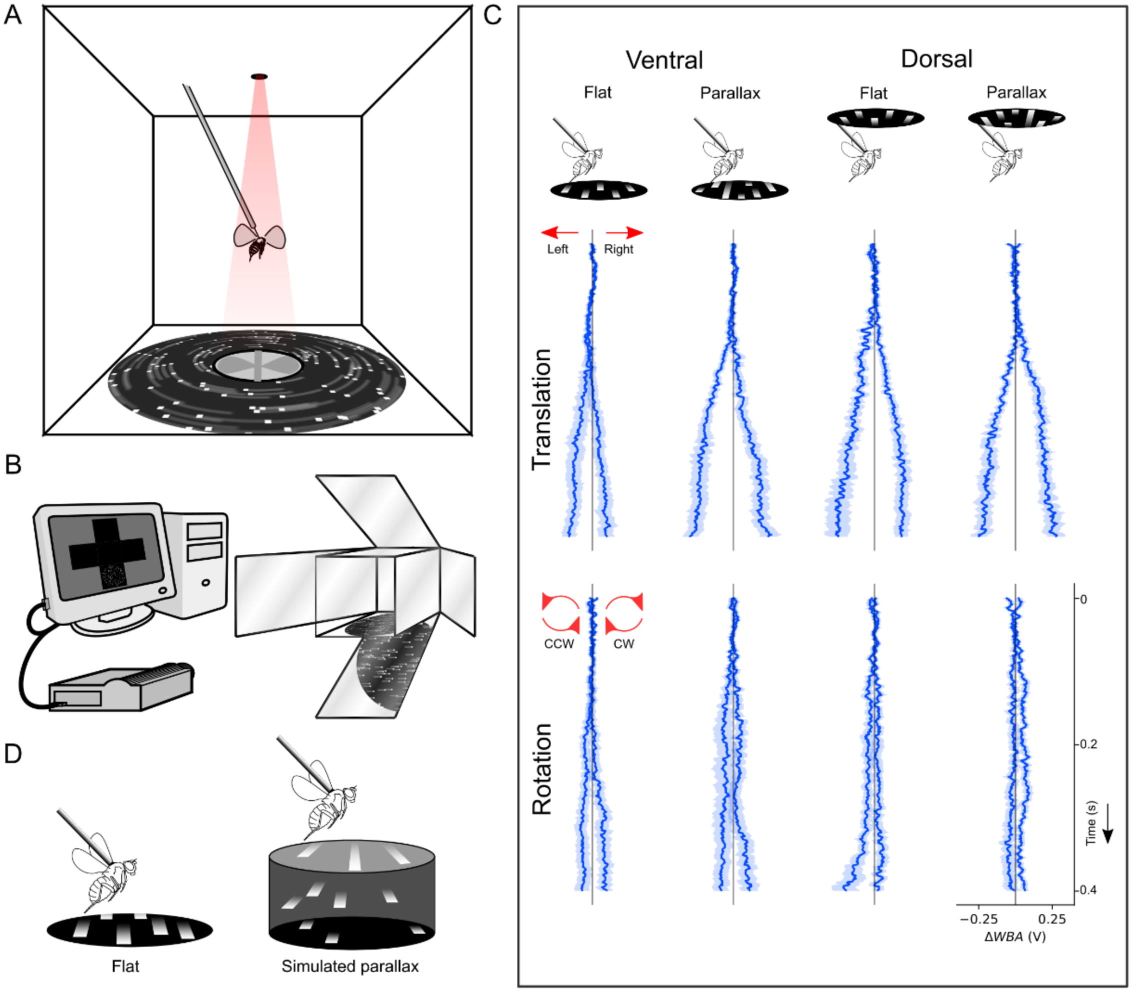

Figure 1.

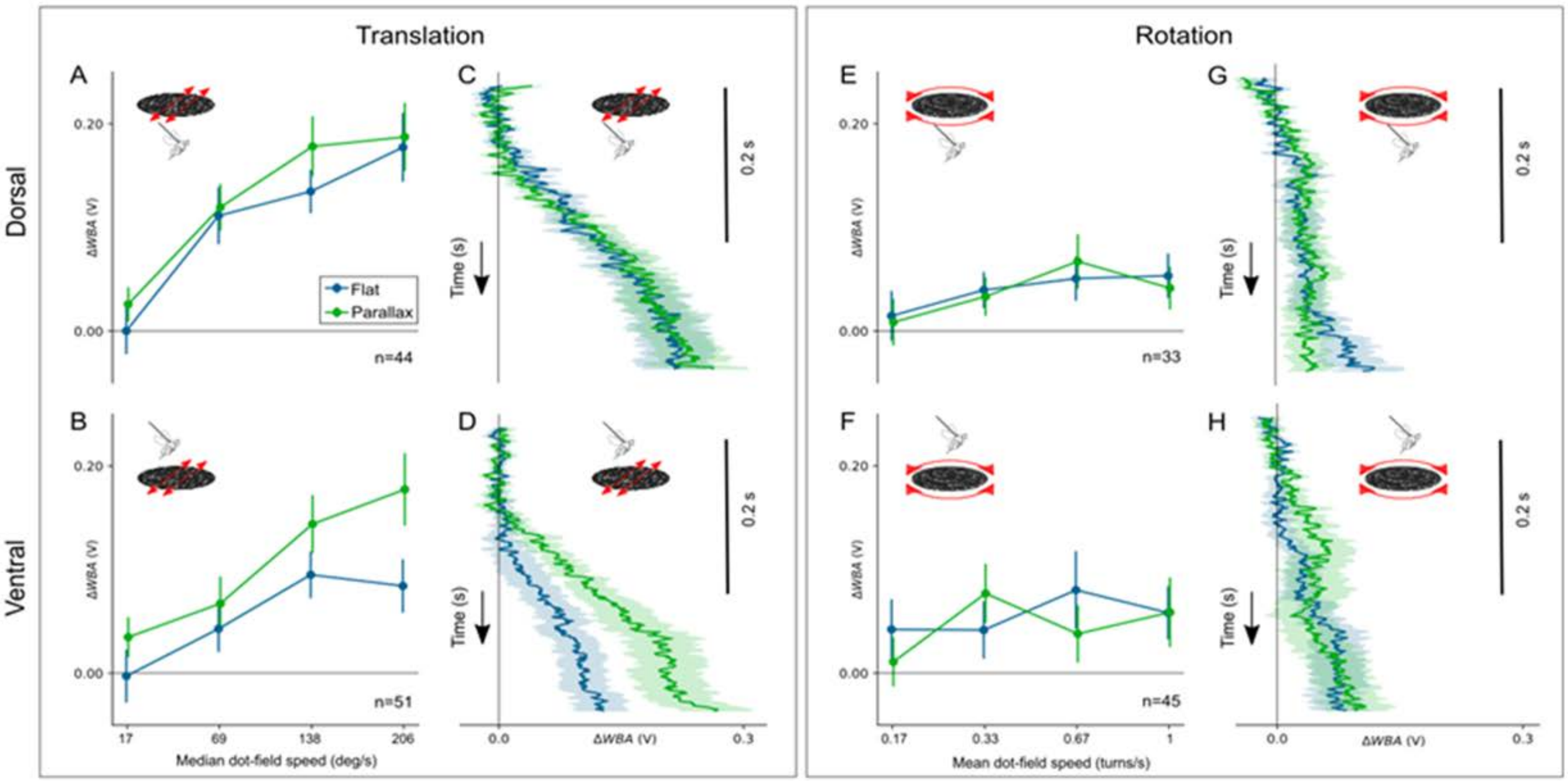
Translation
Rotation
Dorsal
Ventral
A
B
C
D
E
F
G
H
0.20
0.00
ΔWBA (V)
Flat
Parallax
n=44
n=51
n=33
n=45
Time (s)
0.2 s
17
69
138
206
Median dot-field speed (deg/s)
0.0
0.3
0.17
0.33
0.67
1
Mean dot-field speed (turns/s)

Figure 2.

CHAPTER III

STABILIZING RESPONSES TO SIDESLIP DISTURBANCES IN *DROSOPHILA MELANOGASTER* ARE MODULATED BY THE DENSITY OF MOVING ELEMENTS ON THE GROUND

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Abstract

Stabilizing responses to sideslip disturbances are a critical part of the flight control system in flies. While strongly mediated by mechanoreception, much of the final response results from the wide field motion detection system associated with vision. In order to be effective, these responses must match the disturbance they are aimed to correct. To do this flies must estimate the velocity of the disturbance, although it is not known how they accomplish this task when presented with natural images or dot fields. The recent finding that motion parallax in dot fields can modulate stabilizing responses only if perceived below the fly, raises the question of whether other image statistics are also processed differently between eye regions. One such parameter is the density of elements moving in translational optic flow. Depending on the habitat, there might be strong differences in the density of elements providing information about self-motion above and below the fly, which in turn could act as selective pressures tuning the visual system to process this parameter on a regional basis. By presenting laterally-moving dot fields of different densities we found that, in *Drosophila melanogaster*, the amplitude of the stabilizing response is significantly affected by the number of elements in the field of view. Flies countersteer strongly within a relatively low and narrow range of element densities. But this effect is exclusive to the ventral region of the eye, and dorsal stimuli elicit an unaltered and stereotypical response regardless of the density of elements in the flow. This highlights local specialization of the eye and suggests the lower region may play a more critical role in translational flight-stabilization.

Keywords

regional optic flow, dot field density, insect vision, optomotor response, *Drosophila melanogaster*, flight control.

Introduction

Most flies are visual animals with a big part of their heads devoted to the perception and processing of visual information for flight control [1]. Successfully navigating requires them to correct for any deviations from their intended track, which in turn implies distinguishing between self-elicited motion and unintended motion caused by external disturbances. Optomotor responses counteract visual translational and rotational deviations [2], and are refined by the integration of multisensory input [3, 4]. When pushed laterally by a gust of wind, for example, flies countersteer to return to their original route [5, 6].

When evaluating a visual scene for course correction during translation, near objects are more informative than far ones because distant references are less reliable for determining the magnitude of deviations [7-9]. Since objects below the horizon are typically closer to a fly, there is potentially strong selective pressure to evaluate translational disturbances below the horizon in detail [10, 11]. In fact, perception of visual yaw rotation is weighted to stimuli from the upper region of the eye [12], while responses to translation are strongest when it is perceived by the lower region [13]. This functional compartmentalization likely reduces redundancy and enhances accuracy in the perception of self-motion during flight [e.g in different insects: 14-16].

Optomotor responses are further affected by image parameters such as light intensity, contrast, and spatial frequency composition [17, 18]. Within certain ranges, different scenes are accommodated by changes in photoreceptor behaviour [19], or visual processing [20], allowing insects to navigate in a broader set of conditions, but past these responses saturate [21]. The finding that ventral motion parallax mediates steering responses, but dorsal motion parallax does not [22, 23], raises the question of whether flies evaluate other image statistics differently between regions of the eye. In a similar fashion to parallax, the density of moving elements in natural scenes may vary predictably by region, conveying information such as spatial structure and contour distribution in the surroundings [7], in addition to the magnitude of perturbations. To determine the regional effect of element density in the optic flow, we measured the corrective steering responses of *Drosophila melanogaster* to sideslipping dot fields, with different number of elements, across the dorsal and ventral regions of the eye.

Methods

Experimental subjects

We measured steering responses from 50 female *Drosophila melanogaster* 3-5 days after eclosion. The flies came from our lab colony where they were fed with Instant *Drosophila* Medium (Formula 4-24®, Carolina Biological Supply) and kept under a 12L: 12D cycle at 21ºC. We tethered cold-immobilized flies by glueing them to a tungsten rod by the mesonotum. They then recovered for at least 30 minutes in the dark while holding a piece of paper to avoid triggering the tarsal reflex [24]. Upon recovery, we placed each fly in the centre of a projection arena and removed the paper to elicit flight. We tested each fly only once to prevent experience-biased responses.

Visual stimuli

We back-projected onto a 200 mm Perspex cube [9] to display moving dot fields in a 90º diameter disk, directly above or below the fly [23] (figure 1A-C). We used dark-adapted flies in a dark room to ensure they perceived only light from the stimulus. Each experiment consisted of 10 open-loop, randomly ordered trials of white square-shaped dots of equal size, moving laterally on a black background. These were interspersed by closed-loop segments of bar-fixation, using a white bar on a black background, which generates robust flight and standardizes the behavioural state at the beginning of each trial [25, 26]. Dot fields assumed one of 5 levels of density of moving elements (0.4–16.8%), moving coherently left or right, at a constant angular speed (216º/s) for 0.8 s, resulting in a transit time of 0.41 s for individual elements moving along the disk's equator. Densities of moving elements were measured as the mean relative fraction of the stimulus surface covered by dots. Individual dots subtended a maximum of 2.86º on the visual field of the fly, their placement was random and overlapping was allowed. The range of spatial and temporal frequencies across treatments remained constant (figure 1D).

Steering responses

Tethered flies were held at the centre of the arena where an infrared LED placed above cast a shadow of the flapping wings over a dual photodiode below (figure 1B). The difference in the wing-beat amplitude of the left and right wing generated during steering attempts [27] is captured by the dual sensor as a difference in voltage between both sides, due to the change in size of the shadows of the wings [28,29]. Due to the bidimensional nature of the shadows, the wing beat amplitude (ΔWBA) reported by the sensor includes roll and yaw attempts performed by the fly (figure 1E) [6].

Data analysis

Steering responses were normalized based on the highest mean ΔWBA value exhibited by the flies across all treatments within the time window of interest. We used a one-way ANOVA to compare the effect of element density on the amplitude of normalized responses across visual fields (dorsal and ventral) at 0.4–0.7 s after the stimulus was projected (figure 1D). We evaluated significant differences by performing a post hoc pairwise t-test for multiple comparisons, adjusting the p-values using the Bonferroni one-step correction method.

Results

Flies steered with the stimulus direction regardless of the density of visual elements or the region of the eye perceiving motion. Although we found significant differences among responses to element density ($F=6.27$, $p<<0.01$), they occurred regionally. The amplitude of the stabilizing responses to stimuli perceived by the upper eye was not affected by variations in the density of moving elements (figure 2A left, 2D). However in the ventral eye, response amplitude was significantly affected by visual density, being stronger when 4.6 and 8.7% of stimulus was covered by moving elements (figure 2A right, 2D). Although at 4.6% of coverage by moving elements, responses seem to rise faster and peak at a higher amplitude for both eye regions (figure 2B), only in the lower eye this leads to a significant difference with responses elicited by dorsal stimuli (figure 2D). Further, response amplitude to dorsal stimuli was more stable over time across levels of element density (figure 2C) [30].

Discussion

Steering responses to translational flow in fruit flies are stronger when perceived below the equator of the eye [12, 13]. In response to moving gratings, their amplitude depends on the temporal frequency of the stimulus [18, 31]. However, accurate maneuvering in the wild cannot rely only on this parameter due to the wide range of spatial frequencies and contrasts present in natural scenes. Several models attempt to account for such accuracy [e.g. 32, 33], but the actual mechanism remains unknown. Similar to natural scenery, randomly placed dots moving at a constant angular velocity do not provide the fly with a single temporal frequency to modulate their stabilizing responses (figure 1D), yet they respond to faster motion by strengthening corrective maneuvers [23].

Our results suggest that regardless of the actual algorithm used for estimating the magnitude of disturbances in dot fields, the density of moving elements can modulate motor responses, at least when disturbances are perceived on the ground. The strong responses elicited by the ventral region of the eye, within a narrow range of element densities in moving dot fields (figure 2A, C) imply an optimal level of visual elements beyond which a fly may estimate the magnitude of the disturbance less accurately. Interestingly, responses to sideslip presented above the fly suffered no such modulation (figure 2A, C), which could indicate subtle processing differences in dorsal and ventral regions of the eye.

Despite our finding that the density of elements in the dorsal stimuli has no significant effect on stabilizing responses, the time series for this region has a slightly higher amplitude at the same element density at which ventral stimulation produces its strongest response (figure 2B). This may imply flies process element density similarly in both regions of the eye, but its effect on the motor response is reduced in the dorsal region, keeping the system from saturation [21]. If this is the case, translational widefield motion from ventral regions might be scrutinized for parameters such as texture, often irrelevant in dorsal sky views, making the ventral region susceptible to saturation when stimulus features may exceed the range of perception. Flies may further mediate responses by attending to different visual regions, when conditions imply some areas may contain more information [34, 35].

Translational visual cues below the horizon are particularly relevant for controlling ground-speed and correcting changes in position [13, 36, 37]. Blowflies for example, encode motion parallax perceived in this region [22], and fruit flies do the same to modulate their steering responses during flight [23]. Strong sensory adaptation to variations in element density in the dorsal region of the eye could be an adaptive trait for flies traversing perturbed areas and edges of forests, where they would move frequently between structured vegetation and open spaces. Compared to visual elements above the fly that are highly variable, textures below might be more homogeneous and require enhanced sensitivity to small structural changes in order to be used for flight control. Moving from the understory to open areas with the sky as a background dramatically changes brightness and spatial distribution of contrasts above a fly. Exposure to these conditions could produce selective pressure for a regional gain-control mechanism tuned to these parameters [38]. The lower region, on the other hand, suffers a narrower range of variation in image statistics, such as spectral composition, partially because light perceived by this region is mostly reflected off the vegetation [39]. Such conditions could have led to enhanced sensitivity to image statistics conveying structural information, such as the density of moving elements, making the system more susceptible to saturation in this region than dorsally.

Fruit flies and their brachyceran relatives constitute the most ecologically diverse group of flies, many of which feed and reproduce on temporary resources and are forced to move through different habitats to find them [40]. Flight control plays such an important role in the survival of flies that we expect strong selective pressures to act on widefield motion-detecting neurons [41-43]. If our finding represents an adaptive trait associated with high mobility, we will expect similar response patterns in flies with similar habits.

Finally, the levels of element density we tested likely do not saturate responses in free flight, in part because of multisensory integration. The mechanosensory input of halteres and antennae greatly increases the accuracy of the perception of positional and rotational disturbances [3, 4], probably minimizing the effect of changes in element density. Nonetheless, our results provide additional information on how egomotion is perceived and treated regionally from the perspective of vision.

Data accessibility

Data are available from the Dryad Digital Repository:

https://doi.org/10.5061/dryad.vx0k6djq7

Authors' contributions

C.R. helped to develop the research question, carried out all of the laboratory work involved in the data collection, performed the data analysis and drafted the manuscript. J.T. helped to develop the research question, provided logistic support during the time the first author was collecting data and coordinated the study. J.T. also helped by reviewing the manuscript and helping shape the final document. Furthermore, J.T. provided the facilities and equipment necessary to run the experiments and collect the data. Both authors gave final approval for publication and agree to be held accountable for the work performed therein. C.R. and J.C.T. designed the experiment. C.R. collected and analysed the data. C.R. wrote the first draft of the manuscript and J.C.T helped correct it. Both authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests

We declare we have no competing interests

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Figure Captions

Figure 1. (A) Projection arena used to present stimuli to specific regions of the visual field on flies. (B) Tethered flies held under an IR light at the centre of the arena cast a shadow over a sensor below. Changes in wingbeat amplitude (ΔWBA) alter the size of the shadow of each wing and are registered by the sensor as voltages. Differences in amplitude between

both wings represent steering attempts. (C) Responses to regional stimuli were elicited by exposing the flies to laterally-moving dot patterns projected on the upper or lower faces of the projection arena. (D) Contrast as a function of the temporal and spatial frequencies in the stimuli. Coloured regions represent limits of perception for spatial (blue), and temporal (red) frequencies in flies. (E) Mean steering response (solid lines) and standard error of the mean (s.e.m) (colour shading) of 50 flies, elicited by a moving dot field with 4.6% element density, presented to the dorsal and ventral region of the eye at $t = 0$. The grey shading represents the interval over which responses were compared between treatments.

Figure 2. Relative steering responses (ΔWBA) to sideslip disturbances in coherently-moving dot fields as a function of visual element density in dorsal (blue) and ventral (green) visual regions (FOV = 90°, $n = 50$). (A) Means (black) steering responses to dorsal (left) and ventral (right) sideslip disturbances across 5 levels of element density. Violin plots represent the distribution of data within interquartile ranges, along with the median (grey). Mean responses with the same letter are not significantly different (Bonferroni post hoc analysis, $p \leq 0.05$). (B) Heatmaps of mean temporal series at each level of element density in the dorsal (left) and ventral (right) regions of the eye. Dark tones represent relatively stronger responses (ΔWBA). (C) Mean time series of steering responses across levels of element density. Envelopes represent s.e.m and coloured rectangles the time window within which responses were analyzed. (D) Post hoc pairwise t-test for multiple comparisons among both regions of the eye and levels of element density. Bonferroni-adjusted probabilities are represented as shades of grey according to their value. Not significant differences (NS) are represented in light gray.

Figures

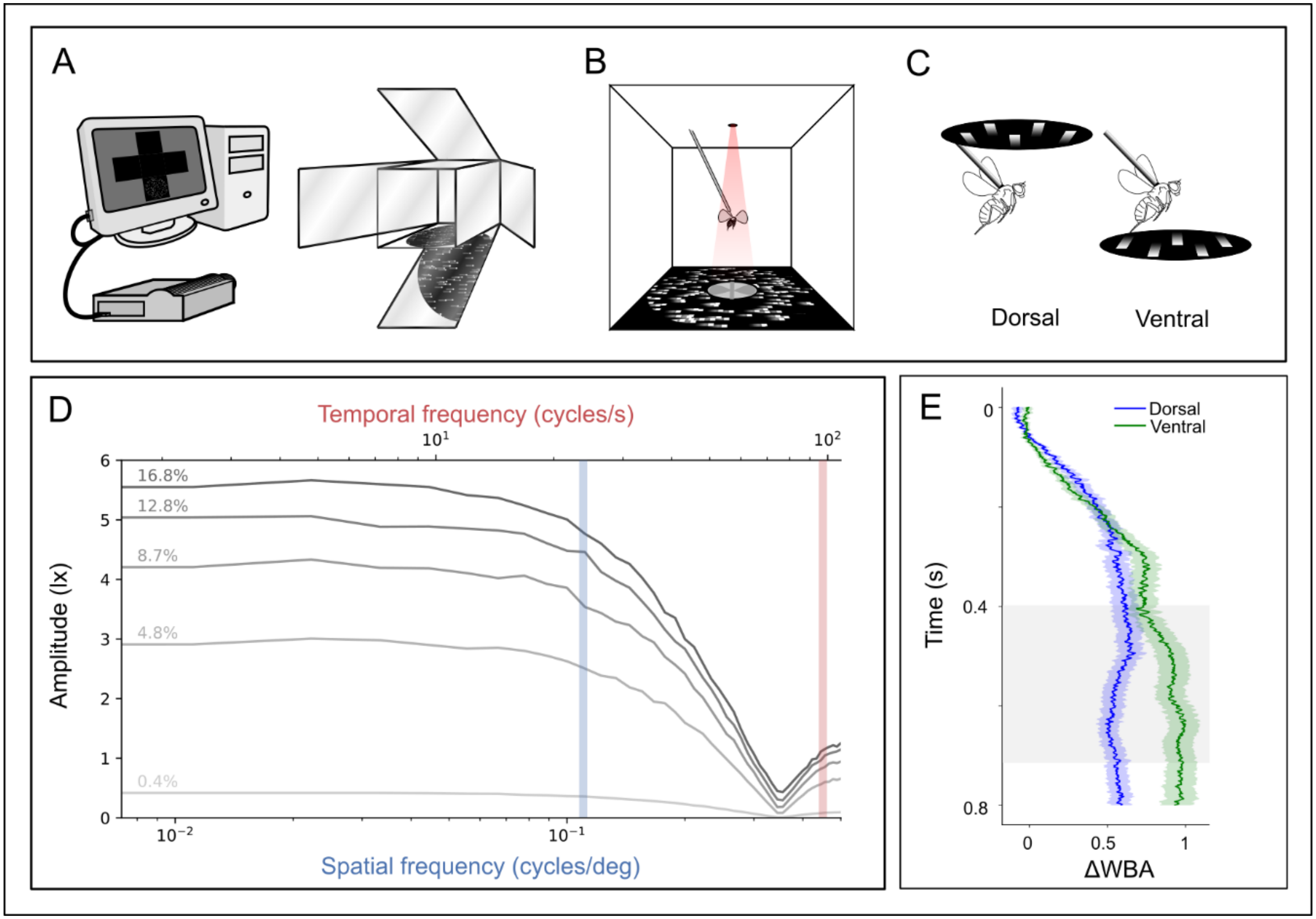

Figure 1.

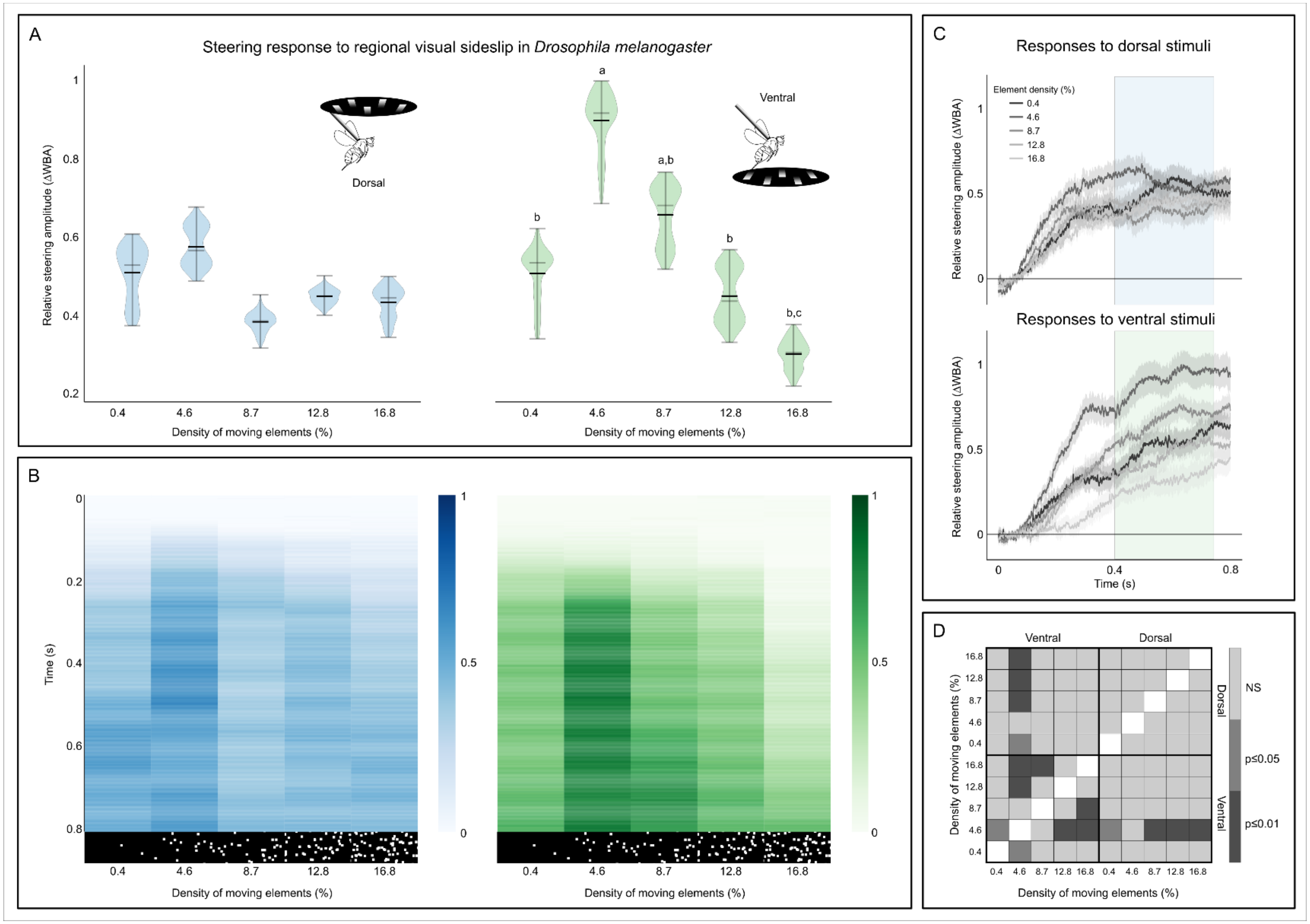
A
Steering response to regional visual sideslip in *Drosophila melanogaster*
Relative steering amplitude (ΔWBA)
1
0.8
0.6
0.4
0.2
Dorsal
Ventral
a
a,b
b
b
b,c
0.4
4.6
8.7
12.8
16.8
Density of moving elements (%)
B
Time (s)
0
0.2
0.4
0.6
0.8
1
0.5
0
C
Responses to dorsal stimuli
Element density (%)
0.4
4.6
8.7
12.8
16.8
Responses to ventral stimuli
Relative steering amplitude (ΔWBA)
Time (s)
D
Ventral
Dorsal
Density of moving elements (%)
NS
p≤0.05
p≤0.01

Figure 2.

CHAPTER IV

HABITAT STRUCTURE AND STABILIZING RESPONSES IN FRUIT FLIES: THE MODULATORY EFFECT OF THE SPATIAL LAYOUT OF VISUAL ELEMENTS ACROSS SPECIES OF DROSOPHILIDS.

Abstract

Flies are among the most maneuverable and fast-flying insects. Their ability to sort obstacles and avoid collisions during high-speed flight depends on the accuracy of motor responses that help them stabilize their flight. Upon the visual perception of a disturbance, a fly must be able to elicit a fast, yet accurate stabilizing response to recover its position, otherwise it would lose control and risk getting killed. The tremendous processing burden imposed on the small brain of a fly has led, over evolutionary time, to optimizations in the way visual information is processed. One of the most important mechanisms adopted is the parallel processing of visual input by separate regions of the eye specialized in the evaluation of particular aspects of motion. While the dorsal region of the eye focuses mostly on detecting changes in direction, the ventral focuses on the perception of translational disturbances. However, unlike rotation, optic flow during translation conveys not only information about self-motion but also about depth and spatial distribution of visual elements, that is the physical structure of the environment. Recent findings show that the ventral region of the eye in *Drosophila melanogaster* is in fact, sensitive to motion parallax and density of moving elements; parameters that convey structural information about the surroundings and can vary dramatically between habitats. However, it is not known whether species of fruit flies adapted to different environments have tuned sensitivities to these parameters, and if that tuning is reflected in the dynamics of their stabilizing responses. To answer this question we evaluated the effect of the density of elements in motion above or below, on the stabilizing responses of three species of fruit flies performing tethered flight in a virtual reality environment. We measured steering responses in *D. melanogaster*, *Z. indianus* and *D. mojavensis*. The first two are cosmopolitan species native to South African habitats with dense vegetation, while the third is endemic to arid regions in the Southwest of the US. We found that the stabilizing responses are modulated in a similar pattern in the three species, by the density of moving elements during ground sideslip. However, the range of responsiveness to element density in the desert species is narrower than in the other two, and its response to dorsal stimuli is inhibited shortly after being elicited. Our findings suggest that the dorsoventral differentiation in the processing of structural parameters, along with the tuning of responses to a particular level of density during sideslip disturbances, are traits associated with the general structure of the visual environment of a fly, regardless of particular details of its habitat. However, the presence of dorsal inhibitory processes and a narrower ventral range of sensitivity to density in the desert fly, strongly suggest these as adaptations to the visual homogeneity of barren environments. Based on these findings, it is likely that subtle modifications in the pattern of responsiveness of the visual system during aerial navigation could allow flies to navigate in habitats completely different visually, opening a door to diversification.

Keywords

element density, insect vision, stabilizing steering, optic flow, *Drosophila melanogaster*, *Drosophila mojavensis, Zaprionus indianus*, fruit fly, African fig fly, vinegar fly.

Introduction

Small flies traversing cluttered environments at high speeds can be easily veered off-course by external agents, such as gusts of wind. In order to minimize the risk of unexpected collisions or predatory attacks, they must elicit a fast and accurate correction to such disturbances. Such corrections are called stabilizing responses and are countersteering maneuvers that bring the fly back to its original position or orientation (Gotz, 1975; Heisenberg and Wolf, 1988). Stabilizing responses are based mostly on visual information (Koenderink and van Doorn, 1987), but can be strongly modulated by mechanosensory input (Budick et al., 2007; Frye and Dickinson, 2004). In order to match the strength of the disturbance to be corrected, the visual system of the fly infers its magnitude using cues from the optic flow (Dahmen et al., 2001; Eckert and Zeil, 2001; Hausen and Egelhaaf, 1989; Srinivasan and Zhang, 2000), and adjusts the response accordingly. While this looks like a simple solution, it has its own set of problems. One of them comes from the restrictions imposed by the small brain of a fruit fly, which limits the neural resources available to process the vast amounts of visual information acquired during a positional disturbance (Wark et al., 2007). Additionally, the visual environment of a fly is anisotropic, implying that different regions in the field of view provide different cues about self-motion, and not all of these cues convey the same information (Dahmen et al., 2001). Flies overcome these limitations in part by optimizing the processing of visual information through functional regionalization of their eyes (Duistermars et al., 2007).

Flies respond to translation more readily when perceived below the horizon (Krapp and Hengstenberg, 1996; Mazo and Theobald, 2014), this is understood as a result of the tuning of the visual system to the characteristic nearness of objects below the horizon, which makes them better sources of information about self-translational motion than far ones (Krapp and Hengstenberg, 1996). Additionally, translational vectors extending along different axes of motion seem to be further segregated into separate streams within the same region of the eye, making stabilizing responses to sideslip independent from forward or backward motion (Theobald et al., 2010b). However, flies are not only more responsive to translational visual cues below them during flight, they also seem to extract more information from those cues when they are perceived by the ventral region of their eyes, instead of the dorsal. In the blowfly *Calliphora vicina* for example, specialized neural elements that extend downward to the lower region of the eye, encode motion parallax (Longden et al., 2017), although it is not known yet how the depth information encoded is used during the generation of stabilizing responses in blowflies. On the other hand motion parallax has a strong effect on stabilizing responses in *D. melanogaster* (Cabrera and Theobald, 2013), although its effect is restricted to the ventral region of the eye (Ruiz and Theobald, 2020). Interestingly, responses generated by this region are also affected by the density of elements moving during a disturbance (Ruiz and Theobald, 2021). Since both of these parameters convey information about the layout of visual elements in the surroundings, their selective processing by the ventral region of the eye suggests that obtaining this type of information from below the fly is critical during navigation and might be a common trait among fruit flies.

Regardless of the habitat in which they evolved, fruit flies are exposed to regularities in the upper and lower regions of their field of view, caused by predictable patterns in image statistics characteristic of natural scenes (Dong and Atick, 1995; Tolhurst et al., 1992; van Hateren, 1997). While the magnitude of these differences can vary throughout different habitats (Dyakova et al., 2019; van der Schaaf and van Hateren, 1996), they are prevalent enough to have played a critical role in the evolution of vision in flies. The wide range of natural histories present in the family Drosophilidae, which includes species adapted to habitats as structurally different as forests and deserts (O’Grady and DeSalle, 2018), makes them excellent candidates for studying how flies have adapted to the perception of disturbances in motion across different habitats.

To determine whether fruit flies, adapted to different visual environments, process image parameters across regions of the eye similarly, we measured stabilizing responses in the vinegar fly *Drosophila melanogaster*, the desert-dwelling fruit fly *D. mojavensis*, and the African fig fly *Zaprionus indianus*, when presented with sideslipping dot fields, with varied densities of moving elements across both regions of the eye.

Methods

Experimental subjects

We measured stabilizing responses from lab-reared vinegar flies *Drosophila melanogaster* and *D. mojavensis sonora* within 3-5 days of their eclosion, and wild-caught African fig flies *Zaprionus indianus* (figure 1A). We kept lab-reared flies at 21°C under a 12 L: 12 D cycle, and fed them standard medium. We put wild-caught flies under the same conditions, for at least 24 hours before using them in experiments. We tethered 50 flies per species by cold-immobilizing and glueing them to a fine tungsten rod by the mesonotum. These flies then recovered upside-down in a dark room for at least 30 minutes, while holding a small piece of paper to keep them still. We tested recovered flies by placing them at the centre of the projection arena and removing the piece of paper to trigger flight. We tested each fly only once to prevent bias in responses caused by experience.

Visual stimuli

We back-projected onto the upper or lower surfaces of a 200 mm Perspex cube to display moving dot fields in a 90º diameter disk (figure 1B, C). The experiments were performed in a dark room, ensuring that the flies at the centre of the cube were perceiving light only from the stimulus. Tethered flies were presented with 10 open-loop, randomly ordered trials during which white square-shaped dots of equal size were moving coherently left or right on a black background at a constant angular speed (216º/s) for 0.8 s. Each dot subtended a maximum of 2.86º on the visual field of the fly and has a random location in the dot field, allowing overlapping. We used closed-loop segments of bar-fixation between the trials to standardize the behavioural state of the flies at the beginning of each trial [Heisenberg and Wolf, 1979; Reichardt and Wenking, 1969]. Flies were presented with dot fields that had one of five levels of densities of moving elements (0.4–16.8%), measured as the percentage of the stimulus area covered by dots (figure 1D left). The range of spatial and temporal frequencies remained constant across densities of moving elements (figure 1D right).

Steering responses

Tethered flies held at the centre of the arena were illuminated from above with an infrared light, casting a shadow of the flapping wings over a dual photodiode below (figure 1E left). Steering attempts generate a difference in the wing-beat amplitude of both wings (Gotz, 1968) changing the voltage on each side of the sensor differentially (Frye and Dickinson, 2004; Gotz, 1987) (figure 1E right). Changes in wing beat amplitude (ΔWBA) reported by the sensor include both roll and yaw attempts due to the bidimensional nature of the shadows of the wings (Theobald et al., 2010a).

Data analysis

In order to increase robustness of comparisons across species, we compared the amplitude of mean normalized steering responses over the last 0.2 s. of the trial using a one-way ANOVA. We evaluated the presence of differences in responses to dorsal and ventral stimuli, across levels of clutter, within and among species. We evaluated significant differences using a post hoc pairwise t-test for multiple comparisons with p-values adjusted using the Bonferroni one-step correction. We used a 2-sample t-test to compare the relative amplitude of steering responses at each level of visual clutter, and a 1-sample t-test to evaluate whether weak mean responses were different from zero. In order to find whether a dorsal response was elicited, we applied a regression model to find the segment of the time series over which the response increases with the highest linearity, starting at 0.03s, which is the minimum time required for the fly to show a motor response to this type of stimulus (Theobald et al., 2010a). We considered highly linearly rising mean amplitudes extending over at least 0.12 s. as positive responses.

We used independent one sample t-tests on each treatment to detect responses that were not significantly different from zero. We evaluated significant differences in steering responses within and across species by performing a post hoc pairwise t-test for multiple comparisons, adjusting the p-values with the Benjamin-Hochberg False Discovery Rate (FDR) method, which offers greater power when the number of comparisons is large, minimizing the occurrence of false negatives.

Results

General behavior

Each of the three species responded robustly to visual translational disturbances by steering syndirectionally with stimuli projected (figure 2); the only exception was *D. mojavensis*, which did not show sustained responses to dorsal stimuli, at any of the five levels of density of moving elements (0.4%: t = 0.18, p = 0.86; 4.6%: t = 0.27, p = 0.79; 8.7%: t = 1.45, p = 0.15; 12.8%: t = 1.04, p = 0.30; 16.8%: t = 1.10, p = 0.27), and failed to respond to ventral stimuli on either end of the range of element densities tested (0.4%: t = 1.46, p = 0.15; 16.8%: t = 1.79, p = 0.08) (figure 2 middle).

Responses to dorsal stimuli

Steering responses to dorsal stimuli in *D. melanogaster* and *Z. indianus*, did not show differences across levels of density of elements moving in the visual field in either species. Despite not being significant, there is a tendency toward slightly stronger responses on the lower half of the range of densities in both species, a similar pattern to the one observed in the ventral region of the eye. However, strong dorsal responses occur over a narrower range of densities in *D. melanogaster* (4.6%) than *Z. indianus*, generating a significant difference in relative amplitude of the response between both species, when moving elements cover 8.7% of the stimulus area (figure 2). Despite the lack of a sustained steering response to dorsal stimuli in *D. mojavensis* during the time frame of the comparative analysis, the time series show the characteristic onset of a steering response similar to that of the other two species, between 0.03 and 0.15 s (figure 3A).

Responses to ventral stimuli

Although in general, variations in the density of moving elements caused a significant effect on steering responses in the three species (F = 6.54, P << 0.001), this effect was restricted to responses elicited by motion perceived below the fly (figure 2, 3B). The amplitude of responses to ground disturbances was significantly affected by the level of element density in the three species tested (figure 3C). The lowest level of element density (0.4%) elicited either a significantly weak (*D. melanogaster* and *Z. indianus*), or no response (*D. mojavensis*) when presented to the ventral region of the eye. Responses peaked similarly across the three species when moving elements covered 4.6% and 8.7% of the dot field, and weakened at higher levels of density (12.8%, 16.8%), being absent in *D. mojavensis* at the highest level. Both *D. melanogaster* and *Z. indianus*, show a similar range of sensitivity to the density of elements moving on the ground during sideslip. Such a range is narrower in *D. mojavensis*.

Discussion

Responses to dorsal stimuli

The robust responses to dorsal stimuli that we found in the Palearctic species *D. melanogaster* and *Z. indianus*, suggest the relevance of upper visual referents for navigation in their habitats. Species associated with dense vegetation have access to a large number of visual elements above them, which could provide information about self-motion during translation. Drosophilids evolving under these conditions could have gained a navigational advantage from retaining responsiveness to translational optic flow on the upper region of their fields of view. Both the African fig fly and the vinegar fly are thought to have originated in the same region in Africa (Karan et al., 2000; Lachaise and Silvain, 2004; Lachaise et al., 1982), apparently in subtropical forests and savannas where moderate to dense canopies are commonplace. Interestingly, we found stronger responses to dorsal stimuli over a wider range of element densities in *Z. indianus* than in *D. melanogaster*. We think it is likely that this character is related to subtle differences in their natural history. Early observations in their original habitat show that both species coexist as part of an ecological succession, in which *Z. indianus* oviposits on figs, after the exit of wasps but before the fruit falls; while *D. melanogaster* colonizes it once it is on the ground (Lachaise et al., 1982). This temporal pattern follows each species' preference for a particular ratio of sugar to protein. While *Z. indianus* prefers ovipositing on fruits with higher contents of sugar, usually ripe fruit on the tree (Matavelli et al., 2015); *D. melanogaster* requires the high protein content, and is attracted to the alcohol present in fallen fruit covered by yeast (Mueller, 1985). Based on this scenario, it is conceivable that early visitors of figs, such as *Z. indianus* are under a strong selective pressure to fly upwards to locate their resource, making translational cues above them more relevant. Late visitors such as *D. melanogaster* on the other hand, can usually locate their resource by flying low among the heaps of fallen fruit. Due to the much wider range of variations in spatial distribution and ranges of contrast generated by canopies against the sky, in comparison to the ground (Frazor and Geisler, 2006), the perception of translational cues from these regions could require a wider perceptual range for these parameters. Hence, it is possible that the enhanced responsiveness of the African fig fly to dorsal stimuli in the middle range of element densities, plays a role in the stabilization of positional disturbances during upward flight, or when the fly is up in the canopy, where motion vectors on the ground are too small to be reliable for the evaluation of self-motion.

Opposed to the Afrotropical species tested, the desert-dwelling *D. mojavensis* showed no sustained responses to dorsal visual stimuli. Such unresponsiveness could be an adaptive trait associated with the visual characteristics of their habitat. Environments with scarce vegetation above the fly, such as deserts, lack visual cues to evaluate translation, besides those below the horizon. Under these conditions, it could be beneficial to devote neural resources to the evaluation of translation specifically on the lower region of the field of view, resulting in weaker or absent responses to dorsal stimuli. Interestingly, we found that *D. mojavensis* does respond to dorsal stimuli but this response quickly disappears. The presence of a positive response is seen as an increase in the amplitude of steering right after the perception of motion (fig 3A, middle row). This onset phase of the response is similar in characteristics to the ones seen in the Paleotropical species (fig 3A, upper and lower rows). This finding suggests that the lack of a stable response in the cactophilic fly could be a derived condition. If this is the case, *D. mojavensis* could have modified its flight behavior simply by shutting down responses to translational cues on the upper regions of its field of view. This would not be surprising, as optomotor responses are flexible enough to allow for the selective inhibition of reflexes, which lets the fly perform certain voluntary maneuvers (Krapp, 2015). Although it is not clear how such a modification could be directly beneficial for survival in deserts, relocating the sensitivity to translational vectors of motion, to regions of the visual field in which they are more likely to be present, would potentially optimize the utilization of neural resources by the visual system.

Responses to ventral stimuli

Our results show that the sensitivity of stabilizing responses, to element density during ground sideslip, observed in the vinegar fly (Ruiz and Theobald, 2021), is a trait also present in both *D. mojavensis* and the African fig fly. Interestingly, despite differences in their natural histories, the three species showed characteristically strong responses at the same level of element density (4.6%), which become weak or absent toward both ends of the range.

The convergence of responses at the same level of element density in the Paleotropical species is hardly surprising given that they both seem to have evolved under the same visual environment. However, the fact that the cactophilic species follows the same response pattern, strongly suggests that the ventral region of the eye is tuned to a set of characteristics of the visual environment that persists across completely different habitats. One important similarity across habitats is that light arriving to the bottom half of the eye is mostly reflected off surfaces, which means that in this portion of the eye flies deal with a narrower range of variation in image statistics than in the dorsal (Prokopy and Owens, 1983). The lower brightness and the higher level of polarization of the light coming from below the fly also causes a strong reduction in the range of variations in contrast compared to the dorsal region (Mante et al., 2005).

While the prevalence of reflected light on the lower region of the visual field forms a pattern that persists across habitats, the arrangement and optic properties of light-reflecting elements grants each environment with unique properties. Complex patterns of vegetation likely generate a mixture of lights and shadows on the lower region of the visual field which is much richer than the one provided by sand on a desert. Such variation in image parameters, particularly in the spatial distribution of contrasts, could have played a role in the retention of sensitivity across a wider range of densities in the Paleotropical species. On the other hand, desert flies navigating a more homogeneous visual environment, with a narrower range of contrast variations on the ground to be used for stabilizing responses, are likely to have reduced the dynamic range of neural elements in that region of the eye (Mante et al., 2005), enhancing their sensitivity to small changes in the density of moving elements.

In *D. mojavensis*, the selective pressures imposed by the habitat on the visual system add to a strong reliance on ground optic flow suggested by its natural history. Mainland populations of the desert-dwelling *D. mojavensis* oviposit only on decomposing patches of the pipe cactus *Stenocereus thurberi* (Ehrman and Wasserman, 1987; Etges, 1989; Etges and Heed, 1987; Heed, 1978; Mangan, 1982; Ruiz et al., 1990). The local abundance of resources provided by this big cactus has allowed these flies to extend their development and reach larger sizes than flies in the more ancestral populations living in Baja California, which specialize in the much smaller *Stenocereus gummosus* (Etges, 1990). Despite the availability of resources derived from the large biomass of *S. thurberi*, the low abundance and scattered distribution of these cactuses, compared to *S. gummosus*, make them more difficult to find (Mangan, 1982; Ruiz and Heed, 1988), forcing the flies to forage long distances to find a new host. These conditions have favored phenotypes with slower development and relatively larger thoraces among mainland forms of *D. mojavensis* (Roff, 1981). Such an adaptive response has been observed in other cactophilic species (Mangan, 1982), and it is linked to an increased dispersal ability (Johnston and Heed, 1976).

While foraging in cactophilic fruit flies is strongly guided by chemical cues (Crowley-Gall et al., 2016; Crowley-Gall et al., 2019), in part due to specific preferences for determined strains of yeast colonizing their host (Date et al., 2017; Fogleman et al., 1981), vision still plays a significant role. *Drosophila mojavensis* for example, has optomotor responses that could be useful in the detection of cactuses in barren environments (Park and Wasserman, 2018). However, in order to find their preferred resources using optomotor anemotaxis, these flies must perform controlled upwind flight, a behavior common in drosophilids (Budick and Dickinson, 2006). In desert environments, where windbreaker vegetation is scarce, this kind of flight is usually performed close to the ground in what is called the boundary layer, which minimizes the effect of strong wind currents (Taylor, 1974). Under these conditions, desert flies such as *D. mojavensis* could have taken advantage of their low flight and absence of structured vegetation above their heads, and focused their responsiveness to drift perceived on the ground, where large flow vectors can provide a much stronger signal to evaluate self-motion (Franz and Krapp, 2000).

Our results also show that steering responses weakened toward both ends of the range of densities in the two Paleotropical species, and were absent in *D. mojavensis*. While the lack of responses in *D. mojavensis* to the lowest level of element density (0.4%) could have been caused by the reduced luminance of the stimulus, it is more likely a result of poor sensitivity to lower levels of density in this region of the eye. This explanation is supported by the presence of an initial response to the same stimulus on the dorsal region of the eye, despite its posterior suppression (figure 3A). Additionally, a similar lack of response occurs at the highest level of density tested, in which luminance is much higher.

This behavior suggests that selective pressures, associated with structural regularities in the visual characteristics of desert environments, could have played a role in reducing the range of element densities over which the ventral region of the eye is sensitive during sideslip disturbances. The relatively weak responses found at the lowest levels of element density in the Paleotropical flies suggest a different scenario. In these two species, dorsal responses were strong enough across densities to rule out the potential effect of reduced luminance on ventral responses to the lowest level. Instead, it is possible that the weakening of responses to ground sideslip, when only few elements provide motion cues, results from inhibitory crosstalk between different motion detecting pathways (Silies et al., 2014). At the lowest element density, the motion of few contrasting elements can result in the simultaneous activation of the large- and small-field motion detection systems, resulting in partial or complete inhibition of stabilizing responses (Fox et al., 2014). In this case, the partial inhibition observed could have been caused by the absence of a textured background moving against the motion of the elements, which would have strongly suppressed responses from the small-field motion detection system (Egelhaaf, 1985a; Egelhaaf, 1985b; Egelhaaf, 1985c; Kimmerle and Egelhaaf, 2000). Furthermore, this is particularly likely to happen during ground sideslip, due to the ventral reach of the receptive field of neural elements in the small-field motion detection system (Aptekar et al., 2012; Reichardt and Poggio, 1976).

Our results show that the modulation of stabilizing responses to sudden ground sideslip, based on element density, is shared by at least three species of fruit flies, one of which is adapted to desert environments. Furthermore, we found that the relative amplitude of stabilizing responses peaks at the same level of element density in the three species studied. This could mean that such a pattern of processing of image parameters and the sensitivity of the lower region of the eye to them, are ancestral traits, instead of adaptations to particular visual environments. Although the phylogenetic relationships of the subfamily Drosophilinae have not been clearly established, many recent reconstructions recover *D. melanogaster* in a clade which diverged earlier with respect to the one containing *D. mojavensis* and *Z. indianus* (Kwiatowski and Ayala, 1999; Tatarenkov and Ayala, 2001; van der Linde and Houle, 2008; Van Der Linde et al., 2010), although an alternative topology has also been proposed (Remsen and O'Grady, 2002). This placement suggests that the sensitivity of the ventral region of the eye to the density of elements moving during sideslip, along with the tuning of responses to a particular level of density, are both ancestral characters that are part of the body plan of these flies. Our results indicate that the evolution of highly specialized natural histories in the family could have been facilitated by subtle changes in the way these traits modulate flight behavior.

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Figures

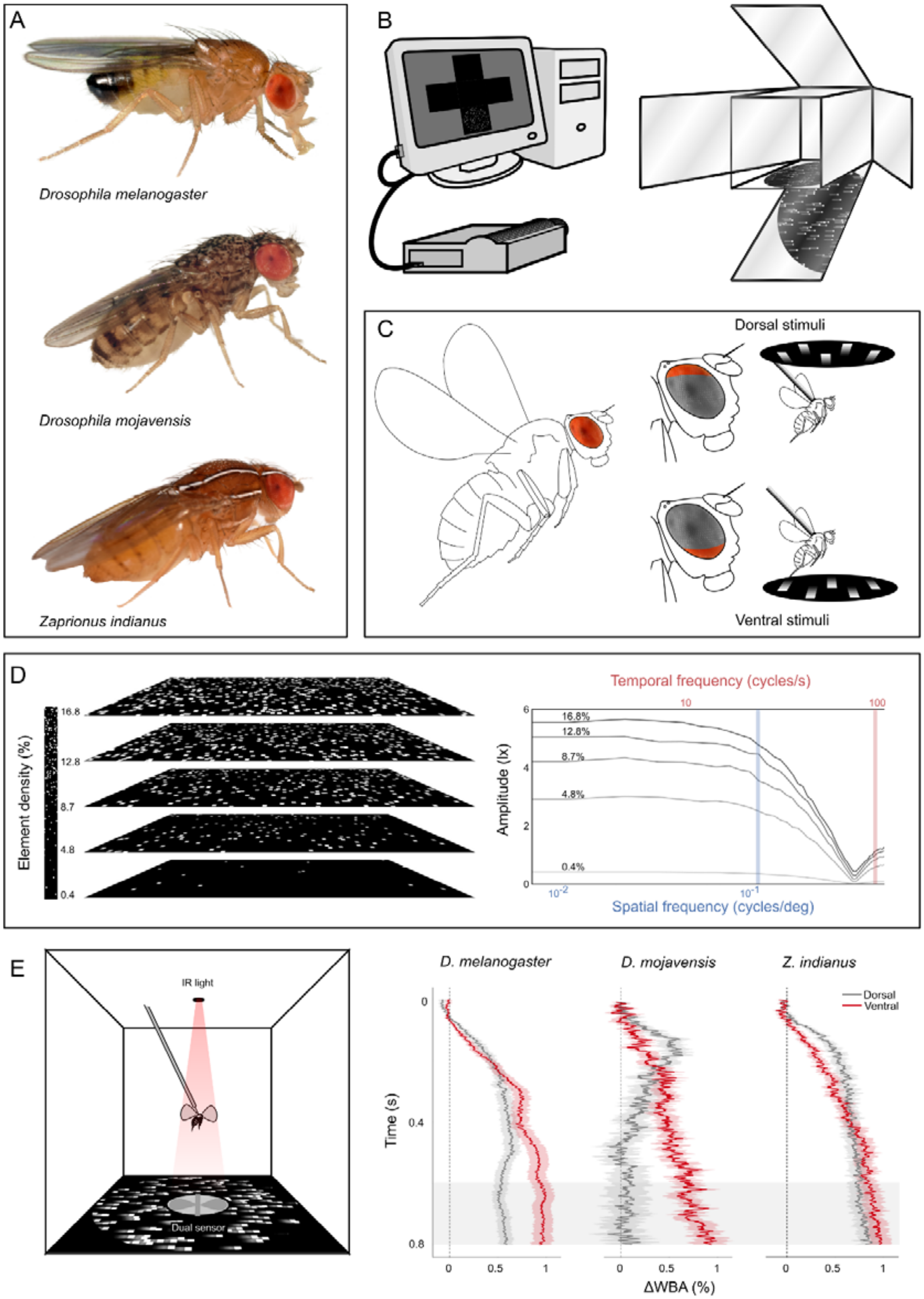

Figure 1. (A) Steering responses were evaluated in three species of fruit flies representing variable levels of phylogenetic relatedness and a range of habitat natural histories. The two

Afrotropical species *Drosophila melanogaster* and *Z. indianus*, the last one which only recently expanded its distribution through America; and *D. mojavensis*, a species endemic to the Southwestern arid regions in the US, which paradoxically is more closely related to *Z. indianus* than to *D. melanogaster*, highlighting the paraphyletic status of the genus Drosophila (photographs by CR). (B) Virtual reality setup used to present visual stimuli to specific regions of the visual field of flies during tethered flight. (C) The body position of a free-flying fruit fly is emulated during tethered flight. The dorsal and ventral regions of the eye perceiving stimuli during tethered flight are highlighted. (D) Dot fields used to present laterally-moving stimuli either above or below the fly. Although the mean brightness of the stimulus increases with element density, spatial and temporal frequencies retaining their patterns of distribution across the treatments. (E) The dual photodiode below the IR-illuminated fly registers changes in the area covered by the shadow of the wings that are translated into time series representing steering responses (ΔWBA). Traces on the right are actual sample responses in each one of the species studied, elicited by both dorsal (gray) and ventral (red) stimuli.

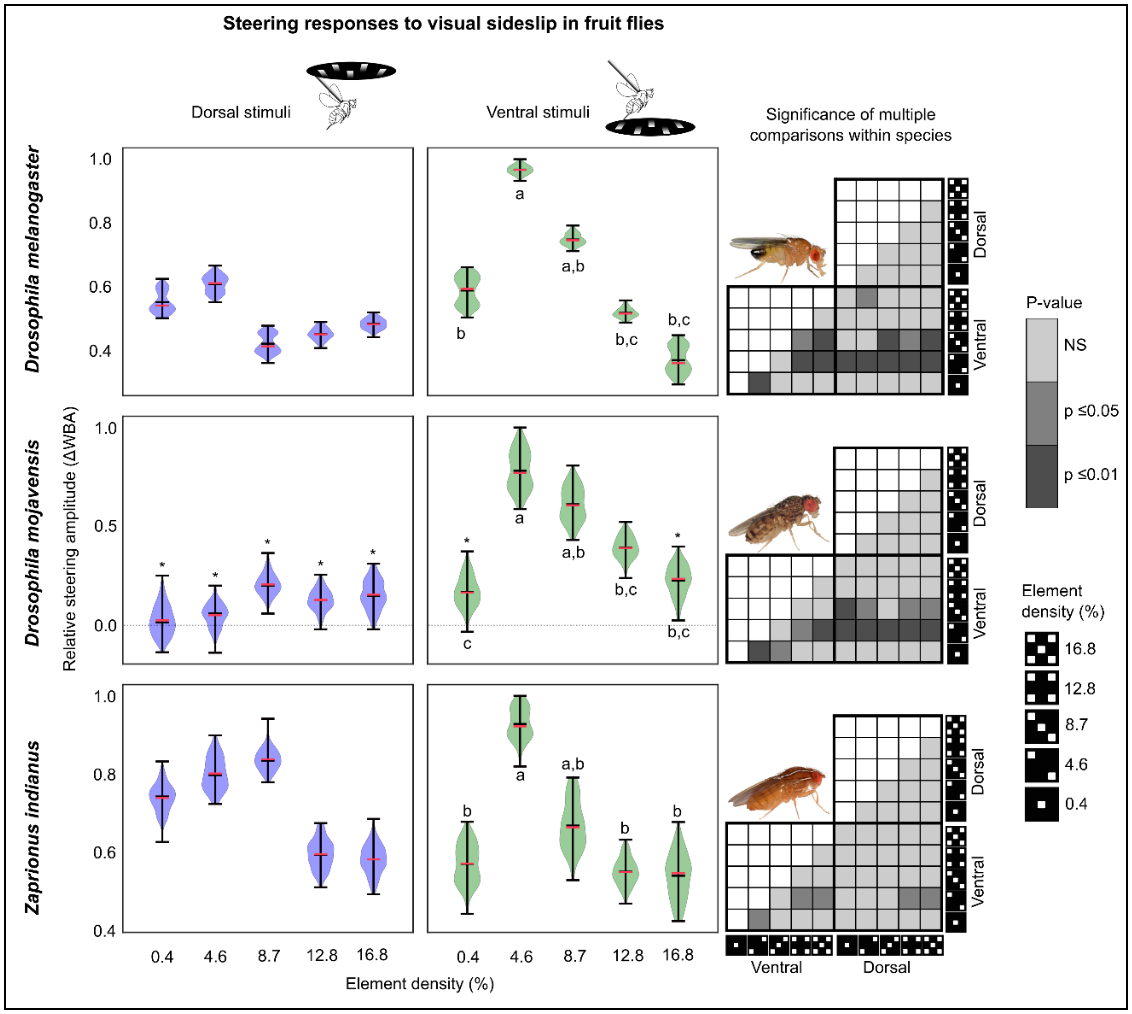

Figure 2. Intraspecific comparison of dorsal and ventral steering responses to visual sideslip in three species of fruit flies. Violin plots represent the distribution of data points within interquartile ranges for each level of element density moving across the dorsal (blue) or the ventral (green) regions of the eye, along with the mean (red) and the median (black) for each species. Grayscale scale heat maps represent FDR-corrected p-values for all comparisons within each species.

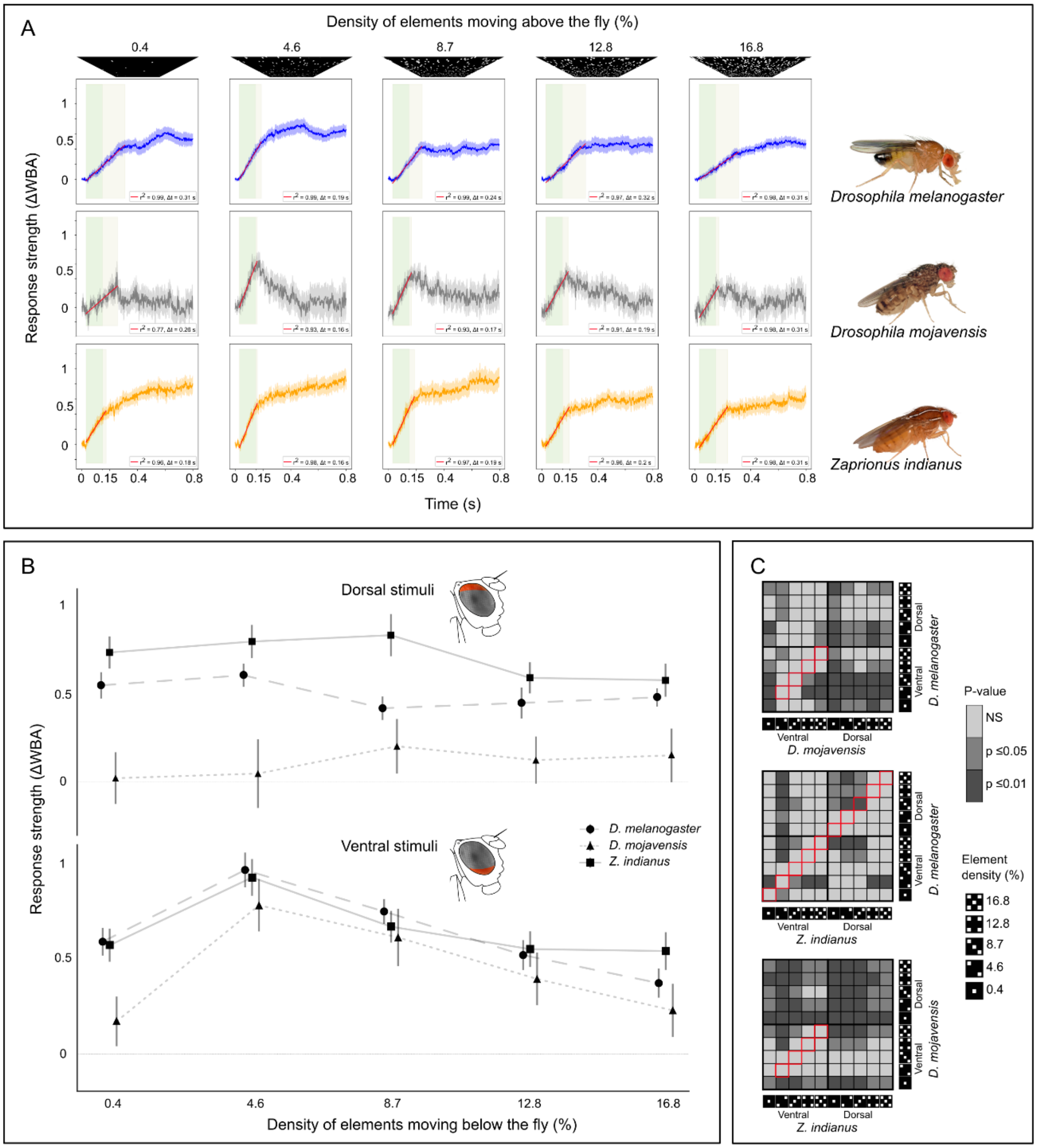

Figure 3. Interspecific comparison of dorsal and ventral steering responses to visual sideslip in three species of fruit flies. (A) Time series of responses to dorsal sideslip. The presence of a response is represented by a robust linear trend of growth in the amplitude of steering (red line) happening between 0.03 and 0.15s after the onset of the stimulus (green region). However, responses often rise for a longer time (Δt) before stabilizing or being inhibited (grey region). (B) Comparison of ventral and dorsal responses among species, at each level of element density. (C).

CHAPTER V

CONCLUSIONS AND FUTURE DIRECTIONS

The study of motor responses to visual stimuli in fruit flies has come a long way since the work of Hecht & Wald (1934), describing the effect that moving stripes have on walking flies. Over the last 60 years, a vast amount of research has helped understand the neuronal basis of motion perception, mostly by characterizing steering responses to moving stimuli in flies. The development of techniques to evaluate steering responses in tethered flies allowed the precise quantification of the effect that subtle changes in visual stimuli have on behavior. However, due to inherent limitations in technology, for a long time, most of the stimuli that could be evaluated were visually simple, usually consisting of contrasting stripes on a rotating drum. Regardless of its simplicity, the implementation of this setup, provided valuable information on the effects of changes in contrast, brightness and spatial resolution, on the steering maneuvers of tethered flies. The knowledge accumulated this way encouraged authors to develop algorithms to explain how the visual input was processed by the fly, in order to elicit responses, and identify neural correlates involved in the process.

With the advent of microcontrollers, it became possible to present more complex visual stimuli such as moving dot fields, at the high frame rates required for flies to perceive fluent motion. However, even though moving dot fields can elicit strong motor responses in fruit flies, just like moving gratings, how flies process motion in them is not completely understood. In part, this is because most of the algorithms that account for the generation of steering responses were developed at a time in which visual stimuli composed by complex moving dot fields were not possible to generate. A clear example of this situation has to do with our understanding of how flies measure stimulus velocity. Research on responses to moving gratings made clear that flies measured stimulus velocity using a combination of two simple parameters, spatial frequency and angular velocity. In other words, flies use temporal frequency to modulate their steering responses. However, the existence of a fundamental temporal frequency is a characteristic of moving gratings. In the wild, flies are exposed to moving visual stimuli containing a wide range of spatial frequencies and velocities, which precludes the use of temporal frequency to generate velocity estimates. Dot fields provide a similar scenario, they contain a whole range of spatial frequencies, making it impossible for the fly to use a fundamental temporal frequency to modulate their steering. Nevertheless, flies manage to consistently modulate their responses according to the characteristics of dot fields, challenging our understanding of the processing of motion.

With the tools we have available now, it is possible to present complex patterns of motion to any region of the visual field of a tethered fly and evaluate its response. These tools are being used to understand how flies manage to process the huge amounts of visual information provided by photoreceptors during the perception of a positional disturbance. In my dissertation, I used these tools to investigate the selective processing of visual input across regions of the fly eye, and explored its potential meaning as adaptive traits, associated with habitat structure.

In chapter II, I explored the regional relevance of depth cues during the generation of stabilizing responses in vinegar flies. My finding that depth cues are only relevant when they appear on the lower region of the visual field opened the possibility that flies evaluate the structure of their habitat differently across eye regions. In this sense, the fact that translational vectors of motion are stronger on the ground (Krapp and Hengstenberg, 1996), could be related to the evolution of a more detailed evaluation of translation in that region.

My results also further our understanding of the ways in which flies optimize the processing of visual information.

Chapter III expands the findings of the previous chapter to include the role of the spatial layout of visual elements in the modulation of stabilizing responses in the vinegar fly. My finding that the spatial arrangement of visual elements affects the amplitude of stabilizing responses, showed that flies craft their responses according to the structure of their surroundings. Furthermore, such modulation is also regional and linked to ground disturbances. These two chapters together strongly pointed at the possibility that during sudden changes in position, flies modulate their responses according to the physical layout of their habitat. Since such layout of elements varies across different habitats, these findings opened the possibility that species adapted to different environments could exhibit different modulation patterns in their responses.

Chapter IV builds off of the previous findings by evaluating the effect of the spatial arrangement of elements on the visual environment of the fly, during the correction of positional disturbances. In this chapter, I compared the modulation of steering responses in two species of fruit flies adapted to dense forests and one that is only found in the Sonoran desert. I found that they share some of the patterns of modulation but do exhibit what could be tuning to their visual environment and natural histories. I discussed these traits in the context of the accepted evolutionary relationships for these species and proposed possible selective advantages of these patterns of modulation.

These three chapters together further our understanding of how flies optimize the processing of visual information relevant to navigation, across habitats with different structural features.

Furthermore, the answers that I found have raised new questions. While relative motion and the spatial arrangement of visual elements in the moving scene provide information about the structure of the environment, these are not the only relevant elements to be considered by the fly during a positional disturbance. Natural environments have ranges of contrasts that follow anisotropies inherent to the presence of the sky and the ground. These ranges vary across environments and throughout the day, as light conditions change. The contribution of these parameters to the navigation of insects are not known in detail. As I have demonstrated here, a detailed study of responses across several species provides critical insight into the evolution of flight. Additionally, it is necessary to evaluate the relevance of the modulatory effects that I found, under free flight conditions. Since tethered flight eliminates critical mechanosensory cues that flies use in the wild, the evaluation of equivalent stimuli during free flight, would provide valuable information on the mechanisms of multimodal integration during flight.

In conclusion, my work has contributed to expanding the understanding of visually-driven behavior in insects by exploring aspects of the processing of visual information during flight in flies.

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