Vision Research 169 (2020) 33–40

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Small eyes in dim light: Implications to spatio-temporal visual abilities in *Drosophila melanogaster*

Ravindra Palavalli-Nettimi*, Jamie C. Theobald

Department of Biological Sciences, Florida International University, Miami, FL 33199, USA

ARTICLE INFO

Keywords:
Low light intensity
Miniaturization
Wide-field motion
Optomotor response
Tuning curve
Sensitivity

ABSTRACT

Fruit flies, *Drosophila melanogaster*, are active over a range of light intensities in the wild, but lab-reared flies are often tested only in bright light. Similarly, scarce feeding during larval stages—common in nature—generates smaller adults, and a wide range of eye sizes not found in well-fed lab colonies. Both dimmer light and smaller eyes limit light capture and have undetermined effects on visual behaviors such as flight. In this study, we used moving sinusoidal gratings to test spatial acuity, temporal acuity, and contrast threshold of female flies of varying eye sizes at different light intensities. We also investigated vision in the smaller and often neglected male fruit flies. As light intensity drops from 50.1 lx to 0.3 lx, flies have a reduced spatial acuity (females: from 0.1 to 0.06 cycles per degree, CPD, males: 0.1 to 0.04 CPD) and temporal acuity (females: from 50 Hz to 10 Hz, males: 25 Hz to 10 Hz), and an increased contrast detection threshold (females: from 10% to 29%, males: 19% to 48%). We find no major sex-specific differences after accounting for eye size. Visual abilities in both small (eye area of 0.1–0.17 mm^2) and large flies (0.17–0.23 mm^2) suffer at 0.3 lx compared to 50.1 lx, but small flies suffer more (spatial acuity: 0.03 vs 0.06 CPD, contrast threshold: 76% vs 57%, temporal acuity: 5 Hz vs 10 Hz). Our results suggest visual abilities of small flies suffer more than large flies at low light levels, possibly leading to size- and light intensity-dependent effects on foraging, navigation, and flight.

1. Introduction

1.1. Light intensity and visual signal noise

The light intensity at which an animal is active can affect several aspects of its life, including foraging, navigation, and mating. Under dim light, some animals continue to extract visual information while others rely increasingly on non-visual sensory modalities (Warrant, 2008a). For flying insects, dim light can reduce the visual ability to detect objects, such as food, obstacles, predators, or mates (for example, (Stöckl et al., 2017a, 2017b)), or hinder foraging activity (Kelber et al., 2006), navigation (Warrant & Dacke, 2011; Warrant, 2008b), flight control, and landing precision (Baird, Fernandez, Wcislo, & Warrant, 2015; Theobald, Coates, Wcislo, & Warrant, 2007). As light intensity falls, performing such behaviors becomes challenging because visual capabilities may be limited by high relative visual signal noise (Warrant & Dacke, 2011; Warrant, 2008b).

A main cause of signal noise in dim light is the stochastic nature of photon absorption. Photon capture follows a Poisson distribution, and thus the degree of uncertainty of photon capture called 'photon shot noise' is given by the standard deviation, $\sqrt{n}$, where n is the number of photons. If the visual system collects n photons and the photon shot noise is $\sqrt{n}$, then the signal-to-noise ratio (SNR) is $\frac{n}{\sqrt{n}}$, or $\sqrt{n}$ (de Vries, 1943). So under low light intensity, SNR is low due to fewer photons being captured by the eye. For reliable vision, high SNR is essential (de Vries, 1943; Rose, 1942). In addition to photon shot noise, intrinsic physiological noise at the photoreceptor level (transducer noise) can further limit SNR (Honkanen, Immonen, Salmela, Heimonen, & Weckstrom, 2016; Lillywhite & Laughlin, 1979; Rieke & Baylor, 1996). The overall signal-to-noise ratio for visual information can thus vary at different light intensities.

Many insects, especially nocturnal flyers, have adaptations to increase their light sensitivity or photon capture and thus improve SNR for visual behaviors. For example, superposition eyes, larger apertures, wider and longer photoreceptors, and shorter focal length can all increase the number of photons captured and thus the sensitivity (Warrant, 2017). Fruit flies, for example, possess neural superposition compound eyes. In this eye type, each point in space is sampled by multiple photoreceptors from different adjacent ommatidia that converge upon the same synaptic units in the brain, which allows for increased sensitivity in dim light. Reliability of visual signals can also be increased by having slow-responding photoreceptors with higher gain

---

* Corresponding author.
E-mail address: rpalaval@fiu.edu (R. Palavalli-Nettimi).

https://doi.org/10.1016/j.visres.2020.02.007
Received 12 December 2019; Received in revised form 27 February 2020; Accepted 27 February 2020
0042-6989/

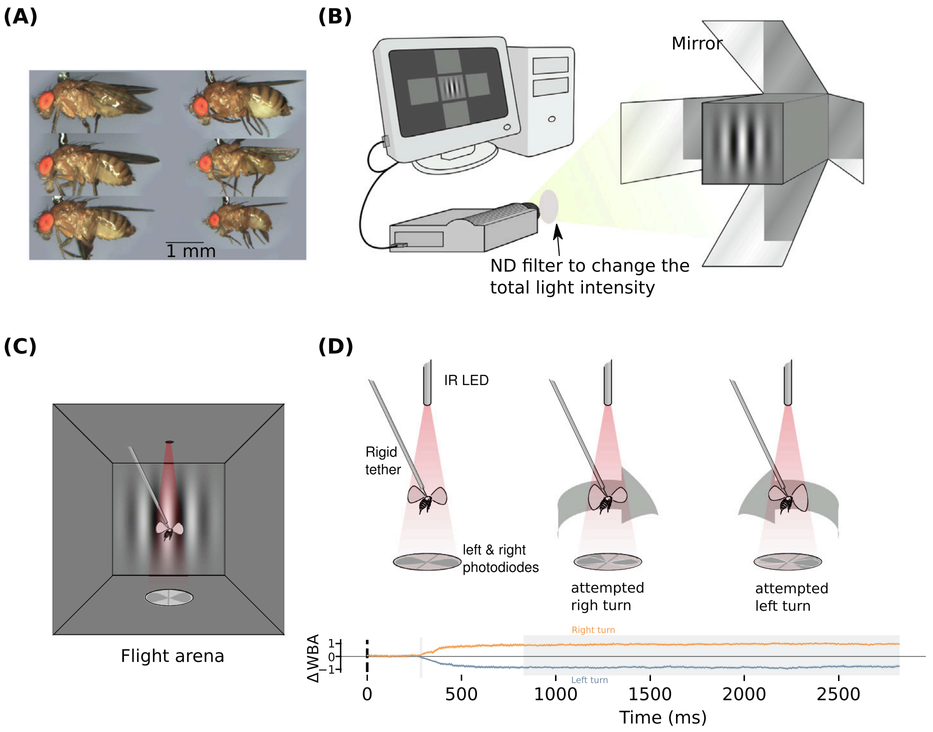

Fig. 1. Virtual reality setup used for measuring spatio-temporal vision in fruit flies. (A) Size variation in *Drosophila melanogaster* (adapted from Currea et al. (2018)). *Ad-libitum* food during late larval stage leads to larger flies but limited larval food availability leads to smaller flies. (B) Computer generated visual stimuli (moving gratings of various contrasts, and spatial and temporal frequencies) are projected onto the flight arena with back projection material on all the walls. A neutral density filter in front of the projector is used to control the total light intensity inside the flight arena. (C) A view from inside the flight arena where a fly is tethered facing the side where the stimulus is projected. (D) Steering response of the fly corresponding to the direction of moving gratings (left or right) is measured using an infrared (IR) light setup. The infrared light casts shadows of each wing onto photodiodes which measure the wing beat amplitude. Left minus right wing beat amplitudes (ΔWBA) gives the steering effort for each wing stroke. An example plot shows the average ΔWBA for 30 flies over a trial. During the bar fixation, ΔWBA is zero. Within each trial, ΔWBA from the last 2000 ms were averaged and subtracted from the average of the first 30 ms (shaded areas). Left and right turns during the trial are indicated.

(Frederiksen, Wcislo, & Warrant, 2008). In addition, neural summation of visual information over the spatial domain can improve vision (contrast sensitivity) in dim light (for example: (Stöckl, O'Carroll, & Warrant, 2016; Theobald, Greiner, Wcislo, & Warrant, 2006; Warrant, 1999, 2008b)). Fast-moving or flying insects need better temporal acuity, and thus under dim light conditions, summation over the temporal domain may trade off with their flight ability (for example, (Sponberg, Dyhr, Hall, & Daniel, 2015; Theobald, Warrant, & O'Carroll, 2010). Hence animals that are active at different light intensities may exhibit an optimum performance at certain preferred light intensities.

1.2. Light intensity and fruit fly activity pattern

Drosophila melanogaster are light sensitive and favor certain light intensity ranges for activity. In a lab environment, they prefer dim light (5–10 lx) for feeding, grooming, and resting, and thus they are considered either crepuscular or diurnal but prefer shaded environments with low light intensities (Rieger et al., 2007). Their choice of certain light intensities can shift their temporal niche. On moonlit nights, or at moonlit intensities in the lab, flies respond by shifting their activity into the night (Bachleitner, Kempinger, Wülbeck, Rieger, & Helfrich-Förster, 2007). Under lab conditions, fruit flies are thought to show a bimodal activity pattern with peaks during early dawn and late dusk (Rieger et al., 2007). However another study showed that under simulated natural conditions where flies were kept in individual tubes ('activity units') outdoors, their activity peaks in the afternoon (Vanin et al., 2012). The authors concluded that under the simulated natural conditions, flies are diurnal rather than crepuscular or nocturnal. Interestingly, under those conditions activity is also temperature-dependent. An interaction between temperature and light is known to affect activity pattern (Majercak et al., 1999) and the time of day can also influence the fruit fly's innate color preference (Lazopulo, Lazopulo, Baker, & Syed, 2019). Whether fruit flies are diurnal or crepuscular has been debated, but these studies agree that flies have preferences for a range of light intensities depending on various other environmental factors. Although fruit flies can be active at different light intensities, how visual abilities vary under these conditions is not systematically studied in this ubiquitous model system, hence this study.

1.3. The effect of size variation in fruit flies on their vision in dim light

Body size in *Drosophila melanogaster* dictates how small eyes are and as a result certain visual abilities (Currea, Smith, & Theobald, 2018). Body size variation is common in fruit flies under natural conditions due to factors such as food scarcity or high larval density (Shingleton, Estep, Driscoll, & Dworkin, 2009). *Ad libitum* feeding in lab colonies, on the other hand, does not lead to the same size variation. It is possible to generate a high variation in body size (1.67–2.34 mm) and eye size (0.07–0.19 mm^2) under lab conditions through limited feeding at late larval stage (Currea et al., 2018). Comparing optical parameters such as ommatidial diameters and interommatidial angles of large and small flies shows that small flies lose more contrast sensitivity than spatial acuity. Behavioral estimates by Currea et al. (2018) have shown that small flies then recover contrast sensitivity by sacrificing temporal acuity through neural summation. Due to size-related structural differences, vision in small flies can be expected to face more challenges under low light conditions. Reduced light and eye size can lead to low SNR for many visual behaviors. But how flies of different eye sizes overcome signal noise at different light levels and how their spatio-temporal visual abilities are affected in dim light is not known.

Fruit flies are sexually dimorphic with males almost half the size of females. Although vision in female fruit flies has been well studied, vision in male flies is often neglected. Studying male fruit fly spatio-temporal visual abilities may thus be useful especially in understanding their role in mating behavior.

In this study we used psychophysics experiments in a virtual reality arena to understand the implications of light intensity and eye size on spatio-temporal visual abilities of female and male *Drosophila melanogaster*. We tested this behaviorally by comparing spatio-temporal visual abilities of large and small female flies under different light intensities. We asked whether male flies have different spatio-temporal visual abilities than females at different light intensities. We also compared males and females in the same range of eye sizes to identify any sex-specific differences in their visual abilities.

2. Materials and methods

2.1. Subjects

Lab-reared *Drosophila melanogaster* were fed standard media and maintained at 21 °C on 12 h:12 h light and dark cycle. To generate flies of varying body and eye sizes (Fig. 1A), we isolated the larvae in a jar without food during their third instar just prior to the wandering stage following Currea et al. (2018). Once the flies eclosed, adults were transferred to a jar with standard media and used for experiments 3–5 days post-eclosion. The flies were cold anaesthetized on a Peltier device and mounted on a rigid tungsten rod (0.02 mm diameter) using UV glue (Bondic) on the mesothorax. To avoid any effects of head movement, their heads were immobilized by gluing to the pronotum (unlike in Currea et al. (2018)). The mounted flies were placed upside down during their recovery. To avoid fatigue prior to testing them, a small piece of paper was placed on their legs to stop them from flapping their wings after recovery.

2.2. Flight arena

Before testing, flies were dark adapted in a room with no lights for at least 20 min. Then each fly was suspended in the center of the arena (Fig. 1B) as described before (Cabrera & Theobald, 2013; Currea et al., 2018). A tethered fly was placed facing the front panel of the arena where moving sinusoidal gratings were projected from a digital projector (Lightspeed designs, frame rate = 360 fps), eliciting optomotor responses. The wing beats of the fly in response to the stimulus were captured using an infrared light emitting diode that casts shadows onto a pair of photodiodes (Fig. 1C). The difference between the left and right wing beat amplitude (ΔWBA) is proportional to the yaw torque (Gotz, 1987; Tammero, Frye, & Dickinson, 2004) and gives the steering effort for the wing stroke (Fig. 1D).

2.3. Stimulus

The grating stimulus can be defined by a sine function: $G(x,\,t) = c\sin(f_s x + f_t t)$, where x is a spatial co-ordinate, t is time, c is Michelson contrast, f_s is spatial frequency, and f_t is temporal frequency. Michelson contrast was measured for the gratings as $\frac{(I_{max} - I_{min})}{(I_{max} + I_{min})}$ where I_{max} and I_{min} are the maximum and minimum intensity of the grating. Spatial frequency refers to the frequency of luminance change over distance, and temporal frequency refers to the frequency of the sinusoidal pattern moving at constant speed. The sine gratings were oriented vertically and the motion was to either the left or right direction.

To measure the spatio-temporal visual abilities of flies, we conducted three psychophysics experiments using moving sinusoidal gratings of 10 different contrasts, and spatial and temporal frequencies in a random order. Each of the gratings was tested moving left and right separately. To estimate spatial acuity we presented 10 spatial frequencies (0.166, 0.125, 0.1, 0.083, 0.066, 0.05, 0.04, 0.031, 0.025, 0.02 Cycles Per Degree (CPD)), at the highest contrast that we could achieve (87%) and a temporal frequency of 10 Hz. Similarly, to estimate the temporal acuity we presented 10 temporal frequencies (0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100 Hz) at 0.04 CPD at the highest contrast (87%). To estimate contrast threshold (and its inverse, contrast sensitivity) we tested 10 different contrasts (87, 76, 67, 57, 48, 38, 29, 19, 10, 0%) at 0.04 CPD and 10 Hz. The spatial and temporal frequency values of 0.04 CPD and 10 Hz were chosen as they were roughly optimal for maximizing turning responses in the temporal and spatial domains respectively. Each experiment consisted of open-loop sequences of sinusoidal gratings for 300 frames interspersed by 200 frames of closed-loop vertical bar fixation. A sampling rate of 1000 Hz (1 sample per ms) was used to collect wing beat amplitude data. During the closed-loop bar fixation, the fly's wing beats controlled the position of the bar, which is known to increase responsiveness to experimental gratings (Heisenberg & Wolf, 1979). Each fly was tested only once and any fly that was not responsive for the whole duration of the experiment was removed from the analysis.

2.4. Light intensity

A neutral density filter in front of the projector was used to control the total light intensity inside the flight arena. For each of the above experiments, we tested flies under different light intensities in a random order. To test the effect of light intensity on spatio-temporal vision in females, we first tested seven different light intensities: 50.1, 16.3, 7.6, 3.9 (close to twilight intensity), 1.8, 0.6, 0.3 (moonlight intensity) lx. Based on the results of this test, to study the effect of eye size on vision, and also spatio-temporal vision in males, we tested them under three of the seven intensities: the highest intensity (50.1 lx), close to twilight intensity (3.9 lx) and moonlight intensity (0.3 lx). The light intensities were measured at the fly's position inside the arena using a light meter (Gossen Starlite 2).

2.5. Eye area measurement

For eye size experiments, one eye of each of the mounted flies was imaged under a stereo microscope under different focus levels. The area of the eye was then obtained from the focus stack using an in-house developed software (Currea et al., 2020, *in prep*). We divided flies into groups of small (smaller than the median eye area) and large (larger than the median).

2.6. Data analysis

The difference between the left and right wing beat amplitude (ΔWBA) was normalized such that a positive value is in the direction of moving sinusoidal grating. Within each trial, the ΔWBA from the last 2000 ms were averaged and subtracted from the average of first 30 ms. We used one-sample *t*-test ($\alpha = 0.05$) to check whether ΔWBA for each stimulus was significantly different from 0. In cases where there was a discontinuity in the responses being significantly different from 0, as at 0.3 lx in Fig. 2A and thereafter, we allowed one such gap and considered the next stimulus value at which the response was significantly different from 0 as a threshold. We used the threshold value for detection of different stimuli to infer spatial acuity, contrast threshold and temporal acuity. We then compared the absolute values of spatial acuity, contrast threshold and temporal acuity across different conditions as in Currea et al. (2018).

3. Results

3.1. Light intensity dependent spatio-temporal vision in female fruit flies

Our first aim was to test spatial acuity, contrast threshold and temporal acuity of female flies under 7 different light intensities. The highest spatial frequency that female flies responded to (spatial acuity) dropped from 0.1 to 0.08 CPD at light intensity lower than 3.9 lx (Fig. 2A). It further reduced to 0.06 CPD at moonlight intensity (Fig. 2A). The average ΔWBA response for different spatial frequencies at moonlight intensity also reduced to almost half that at the highest light intensity (Fig. 2A). The lowest contrast that the flies responded to (contrast threshold) was 0.1 at 50.1 lx, which increased to 0.19 at 1.8 lx and further increased to at most 0.48 at dimmer light intensities, and this slower rate of increase in contrast threshold as light levels drop indicates possible spatial summation (Fig. 2B). The average ΔWBA response for different contrasts at moonlight intensity reduced substantially compared to that at the highest light intensity (Fig. 2B). At light intensities higher than 3.9 lx, the highest temporal frequency that the flies responded to (temporal acuity) was 50 Hz, which reduced to

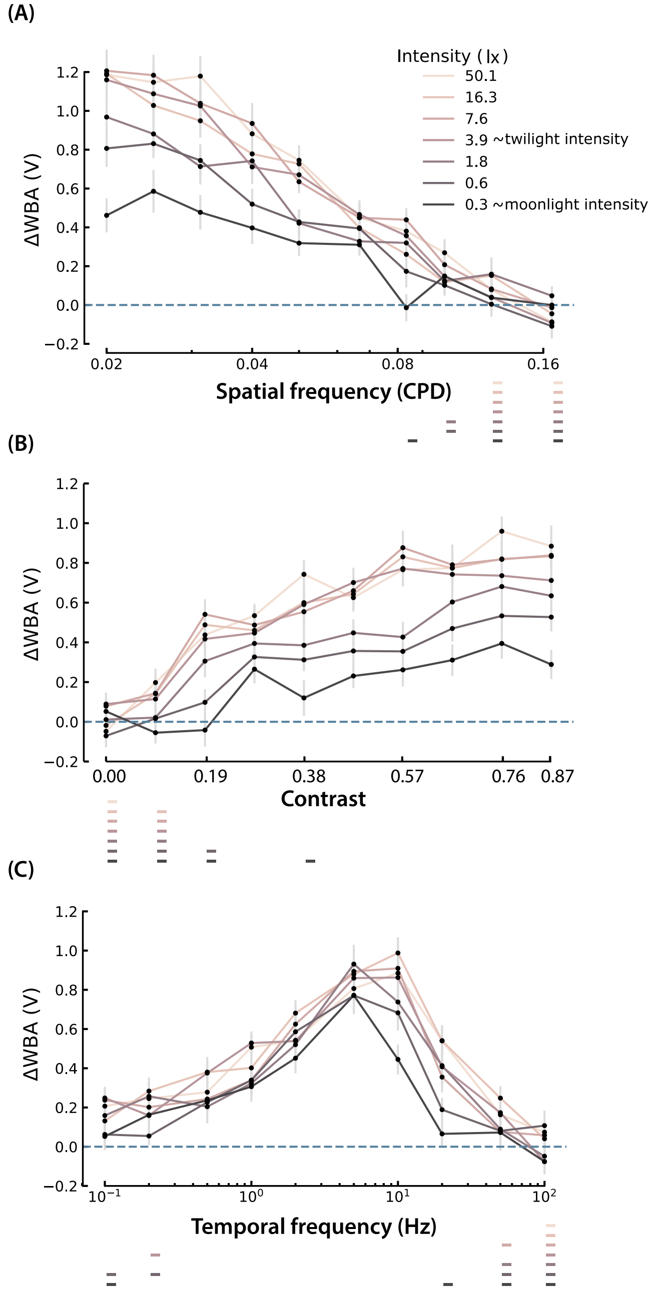

Fig. 2. At light levels lower than twilight intensity, female fruit flies (n = 30) have reduced spatio-temporal visual abilities. (A) The highest spatial frequency that the flies respond to (spatial acuity) is reduced at light intensities less than 3.9 lx. (B) The lowest contrast that the flies can respond to (contrast threshold) is increased at light intensities dimmer than 1.8 lx. (C) The highest temporal frequency that the flies respond to (i.e., temporal acuity) is reduced at 0.3 lx. Differential wing beat amplitudes (ΔWBA) show the steering effort of flies tested in the virtual reality arena. Mean ± SE of ΔWBA is plotted, see methods for details. Dashes below the x-axes indicate sample ΔWBA is not significantly different from 0 (one-sample *t*-test, $p \geq 0.05$).

20 Hz at 1.8 and 0.6 lx, and to 10 Hz at 0.3 lx, indicating temporal summation in dim light conditions (Fig. 2C). The peak response at 0.3 lx also appears to be shifted to a lower temporal frequency of 5 Hz from 10 Hz at 50.1 lx (Fig. 2C), but no such clear peak shift was observed for spatial frequencies and contrasts (Fig. 2A and B).

3.2. Size dependent spatio-temporal vision in female fruit flies under different light intensities

The next aim was to determine whether spatio-temporal abilities of the flies under different intensities are eye-size-dependent. Eye area of the female flies ranged from 0.1 to 0.23 mm^2 (median = 0.17 mm^2). Flies with the eye area of 0.1–0.17 mm^2 were grouped as small and those with an area of 0.17–0.23 mm^2 as large flies. Small flies had similar spatial acuity (0.1 CPD) to large flies at 50.1 lx and 3.9 lx, but a much lower spatial acuity (0.03 CPD vs 0.06 CPD in large flies) at 0.3 lx (Fig. 3 first row). At 50.1 lx, flies showed spatial aliasing at the highest spatial frequency (Fig. 3A first row). That is, they turned in the opposite direction to that of the moving gratings, which is commonly observed in flies at high spatial frequencies (Götz, 1965). At 0.3 lx, compared to large flies, small flies had higher contrast threshold (small flies: 0.76, large flies: 0.57) and lower temporal acuity (small flies: 5 Hz, large flies: 10 Hz; Fig. 3B second and third row). At 3.9 lx, small flies have similar contrast threshold (0.19) but lower temporal acuity than large flies (small flies: 10 Hz, large flies: 20 Hz; Fig. 3C second and third row). However at 50.1 lx, small flies had slightly higher contrast threshold (small flies: 0.19, large flies: 0.1; Fig. 3A second row) and lower temporal acuity (small flies: 20 Hz, large flies: 50 Hz; Fig. 3A third row). With decreasing light intensity, temporal acuity values reduced for both large and small flies (Fig. 3A–C third row).

3.3. Spatio-temporal vision in male fruit flies

We tested spatio-temporal visual abilities of male flies under 3 different light intensities to compare them to females. Male fruit flies had on average smaller eyes (0.14–0.19 mm^2; average = 0.16 mm^2) than females (0.10–0.23 mm^2; average = 0.17 mm^2). Males had similar spatial acuity, contrast threshold, and temporal acuity as females at 50.1 lx and 3.9 lx (Fig. 2 and Fig. 4), but a lower spatial acuity (0.04 CPD; Fig. 4A) at 0.3 lx and much reduced mean response in general (Figs. 2A–C and 4A–C). The peak response at 0.3 lx was clearly shifted to lower temporal frequency of 5 Hz from 10 Hz at 50.1 lx (Fig. 4C).

3.4. Spatio-temporal visual abilities of males and females of the same eye size range

To understand sex-specific differences in fruit fly vision, we compared males and females of the same eye size range (0.14–0.19 mm^2). Median eye area for both sexes was 0.17 mm^2, and mean ± SD were 0.169 ± 0.013 and 0.166 ± 0.015 mm^2 for females and males respectively. At 50.1 lx, males and females had the same spatial acuity, contrast threshold, and temporal acuity (Fig. 5A). At 3.9 lx, males had the same spatial acuity as females, but slightly lower contrast threshold and higher temporal acuity (Fig. 5B). At 0.3 lx, males had roughly similar reduced spatio-temporal visual abilities although female data are more variable in their response to spatial frequencies and contrasts (Fig. 5C).

4. Discussion

Under natural conditions, fruit flies are exposed to a broader range of light intensities and exhibit a wider range of body sizes than in the lab. Here we studied how reduced light intensity and eye size affect spatial acuity, contrast threshold, and temporal acuity in fruit flies. We show that the spatio-temporal visual abilities in both males and females reduce when the light levels are below twilight intensity (Figs. 2 and 4). At moonlight intensity, small flies have more reduced spatio-temporal visual abilities than large flies (Fig. 3C).

4.1. Spatio-temporal vision in fruit flies is light intensity dependent

As light levels drop, there is a need to improve sensitivity more than

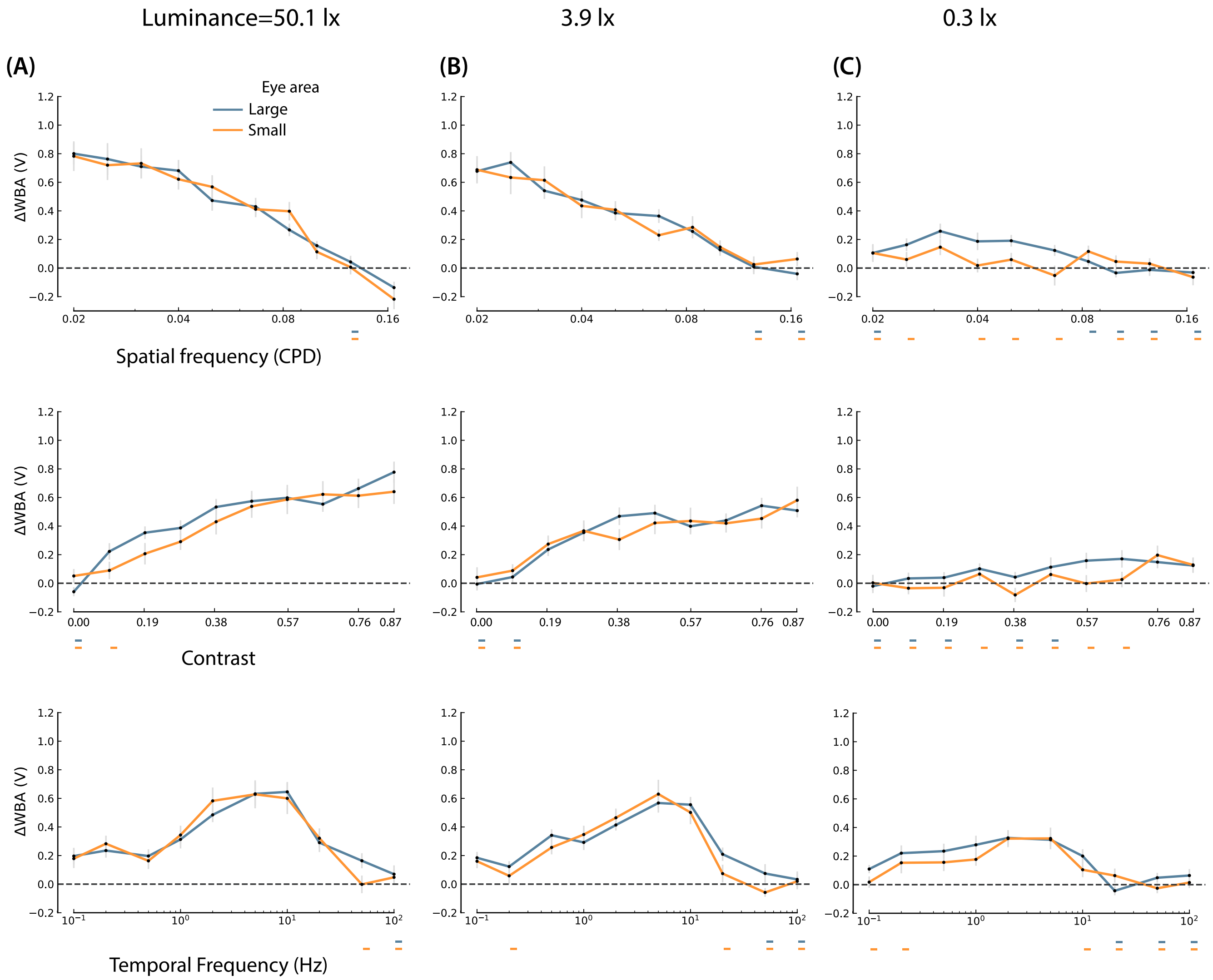

Fig. 3. Different spatio-temporal visual abilities in large and small female flies (n = 25 each) under different light intensities. (A) At 50.1 lx, small flies have the same spatial acuity as large flies but have a slightly increased contrast threshold and reduced temporal acuity. (B) At 3.9 lx (~twilight intensity), small flies achieve same spatial acuity and contrast threshold as large flies presumably by sacrificing their temporal acuity, confirming the results of Currea et al. (2018). (C) At 0.3 lx (~moonlight intensity), spatial acuity and temporal acuity of small flies are reduced more than large flies while contrast threshold is increased. Flies with an eye area in the range of 0.17–0.23 mm^2 were considered large, while 0.10–0.17 mm^2 were considered small. Mean ± SE of ΔWBA is plotted, see methods for details. Dashes below the x-axes indicate sample ΔWBA is not significantly different from 0 (one-sample *t*-test, $p \geq 0.05$).

spatial acuity. Hence animals that are active at low light intensities tend to have optical and neural strategies to improve sensitivity in dim light (Warrant, 1999). Optical strategies such as increased facet diameter can improve sensitivity but simultaneously reduce spatial acuity (Land, 1997). Neural strategies such as spatial and temporal summation can again improve contrast sensitivity but at the expense of spatial and temporal acuity (Warrant, 1999, 2008b). Compromising temporal acuity in dim light is useful because it increases visual SNR, improving contrast sensitivity by suppressing photon noise.

Our data show that at moonlight intensities male and female fruit flies lose both spatial and temporal acuity in order to rescue contrast sensitivity, to an extent. This suggests they cannot detect faster and finer details under moonlight intensities. When comparing males and females of similar body sizes, we find no major sex-specific differences in their spatio-temporal vision (Fig. 5), suggesting the differences between Figs. 2 and 4 are only size-related, not sex-related. Among females, compared to the highest intensity we tested (50.1 lx), while spatial acuity reduced considerably below 3.9 lx, contrast threshold increased below 1.8 lx, and temporal acuity reduced at the much lower moonlight intensity of 0.3 lx (Fig. 2). This implies that as light levels reduce, to improve contrast sensitivity, females sacrifice spatial acuity first, and then temporal acuity. Given the importance of temporal acuity for fast-moving and flying insects, this result is expected. Additionally, peak values for the temporal tuning curve at 0.3 lx shift to lower temporal frequencies compared to that of 50.1 lx (5 Hz vs 10 Hz: Figs. 2C and 4C). Interestingly however, in *Manduca sexta*, optomotor response assays showed that the peak or preferred temporal frequency and time to respond to change in motion does not decrease at low light intensity (Parthasarathy & Willis, 2018) which is in contrast with the results of Stöckl, O'Carroll, and Warrant (2017b) that showed reduction in the peak temporal frequency at low light intensity based on motion-sensitive neuron activity in the Lobula complex of different hawkmoths. In an optomotor behavior assay, while the intensity dependent temporal tuning in flies can help process slower visual motion in dim light, retaining the speed of response in dim light may be more important for hawkmoths such as *Manduca sexta* depending on their visual ecology. Flies may also need to slow down to overcome the loss of temporal acuity at moonlight intensity. Indeed, in dim light, hornets (Spiewok & Schmolz, 2006) and bumblebees (Reber et al., 2015) reduce their flight speed to cope with reduced temporal acuity due to temporal

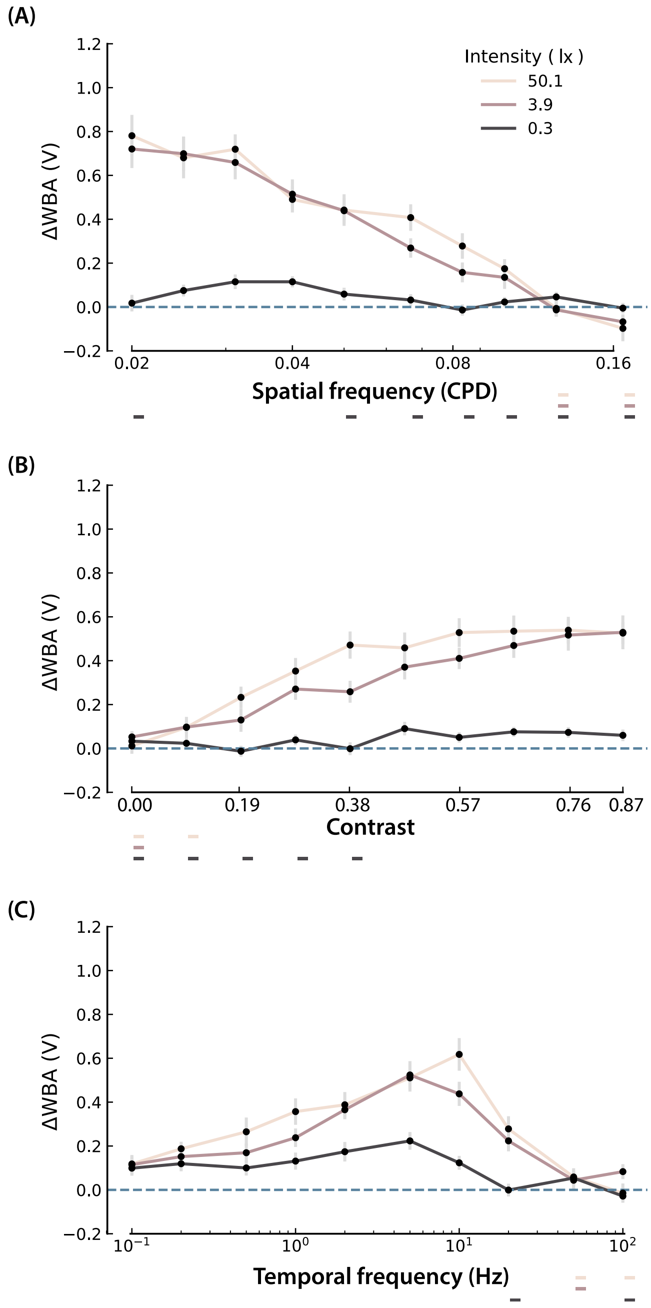

Fig. 4. Spatio-temporal visual abilities of male fruit flies ($n = 31$) are reduced at moonlight intensity. Spatial acuity (A), contrast threshold (B), and temporal acuity (C) changed at 0.3 lx in male fruit flies. Males have an eye area of 0.14–0.19 mm^2. Mean ± SE of ΔWBA is plotted, see methods for details. Dashes below the x-axes indicate sample ΔWBA is not significantly different from 0 (one-sample *t*-test, $p \geq 0.05$).

summation. An exception to this is the nocturnal sweat bee *Megalopta genalis* which does not change flight speed (Baird et al., 2015) but takes more tortuous paths in dim light (Theobald et al., 2007) indicating that it could be using spatial summation rather than temporal summation to regain visual contrast. The relative balance between spatial and temporal summation depends on the ecology and behavioral needs of the animal (Warrant, 1999). For fast flying insects, reduced temporal acuity can affect flight behaviors. As the image motion increases, integration time is reduced, which can lead to increased spatial summation (Klaus & Warrant, 2009). For a given light level, there is an optimum combination of summation to rescue sensitivity (Klaus & Warrant, 2009; Snyder, Stavenga, & Laughlin, 1977). This balance between sensitivity and spatio-temporal acuity may even be adjusted to match the requirements of different visual pathways— such as object detection, looming, and widefield motion detection pathways—in the brain (Stöckl et al., 2017b).

We also find that flies respond more strongly at higher light intensities (Figs. 2 and 4), which could be due to a higher SNR. Changing the gain of the response when the visual cues are unreliable or hard to detect may help minimize metabolic costs associated with neural signaling (Maimon, 2011; Niven & Laughlin, 2008). In fact, such response modulation is known to be state-dependent in flies—higher response gain during flight than walking (Chiappe, Seelig, Reiser, & Jayaraman, 2010; Maimon, 2011; Maimon, Straw, & Dickinson, 2010). During insect flight, octopamine is released (Goosey & Candy, 1980) and can modulate the response properties of motion sensitive neurons (de Haan, Lee, & Nordström, 2012). Activation of the octopaminergic neurons is also involved in multisensory modulation of a fly's innate aversion to small visual objects (Cheng, Colbath, & Frye, 2019). It is possible that the optomotor response in our study may be similarly graded according to reliability or noise from sensory cues during flight. This graded response allows flexibility of visually driven behavioral responses, as seen in our case. Whether spatial summation of signals (in lamina monopolar cells) and temporal summation in dim light could lead to reduced responses in downstream motor neurons via octopaminergic pathway remains to be tested. We also expected responses to reduce at lower spatial frequencies as in (Theobald, 2017), but we found such response reduction only at low intensities (Figs. 2A and 4A). Possibly, high light levels afforded enough visual information to steer, even in response to lower spatial frequencies.

4.2. Smaller flies suffer more than large flies at moonlight intensity

Although there are many studies in the literature on insect low light responses and eye size effects, surprisingly, *Drosophila melanogaster* hasn't been systematically studied in this respect. Smaller insects typically have fewer and smaller ommatidia, lower spatial acuity, and lower sensitivity than larger individuals or species (ants (Palavalli-Nettimi, Ogawa, Ryan, Hart, & Narendra, 2019), aphids (Doring & Spaethe, 2012), bumblebees (Kapustjanskij, Streinzer, Paulus, & Spaethe, 2007; Spaethe & Chittka, 2003; Taylor et al., 2019), bees (Jander & Jander, 2002), butterflies (Rutowski, Gislén, & Warrant, 2009), fruit flies (Currea et al., 2018), moths (Fischer, Meyer-Rochow, & Müller, 2014). Whether the visual abilities in smaller individuals suffer more in dim light, especially in *Drosophila melanogaster,* is not well understood. Due to miniature body and eye size, some insects are apparently restricted to a diurnal lifestyle (Fischer et al., 2014; Fischer, Meyer-Rochow, & Müller, 2012; Fischer, Müller, & Meyer-Rochow, 2011). Smaller stingless bees, for example, limit their activity to brighter times of the day (Streinzer, Huber, & Spaethe, 2016). We can thus expect that larger flies may similarly have an advantage in dim light conditions.

As expected, we found that at moonlight intensity small flies have lower spatial and temporal acuity, and a higher contrast threshold (Fig. 3C). The lower spatial acuity of small flies at moonlight intensity could be due to low SNR and a high degree of neural summation in dim light. Photon shot noise in small flies with smaller lenses and less photon capture is similar to the photon noise in dim light. The neural adaptations for dim light, such as spatial and temporal summation, might help small eyed flies recover their vision to an extent. Hence at dimmer intensity, small flies may need to rely more on summation than large flies and thus have lower spatio-temporal acuity than large flies. As light intensity reduces from 50.1 lx to 0.3 lx, the extent of temporal summation (evident from temporal acuity values) steadily increases (Fig. 3A–C last row) as predicted theoretically (Klaus & Warrant, 2009). At twilight intensity, small flies are able to maintain a similar contrast threshold as large flies due to temporal summation, consistent with findings of an earlier study (Currea et al., 2018). However at a brighter intensity of 50.1 lx, temporal summation may not be necessary and thus

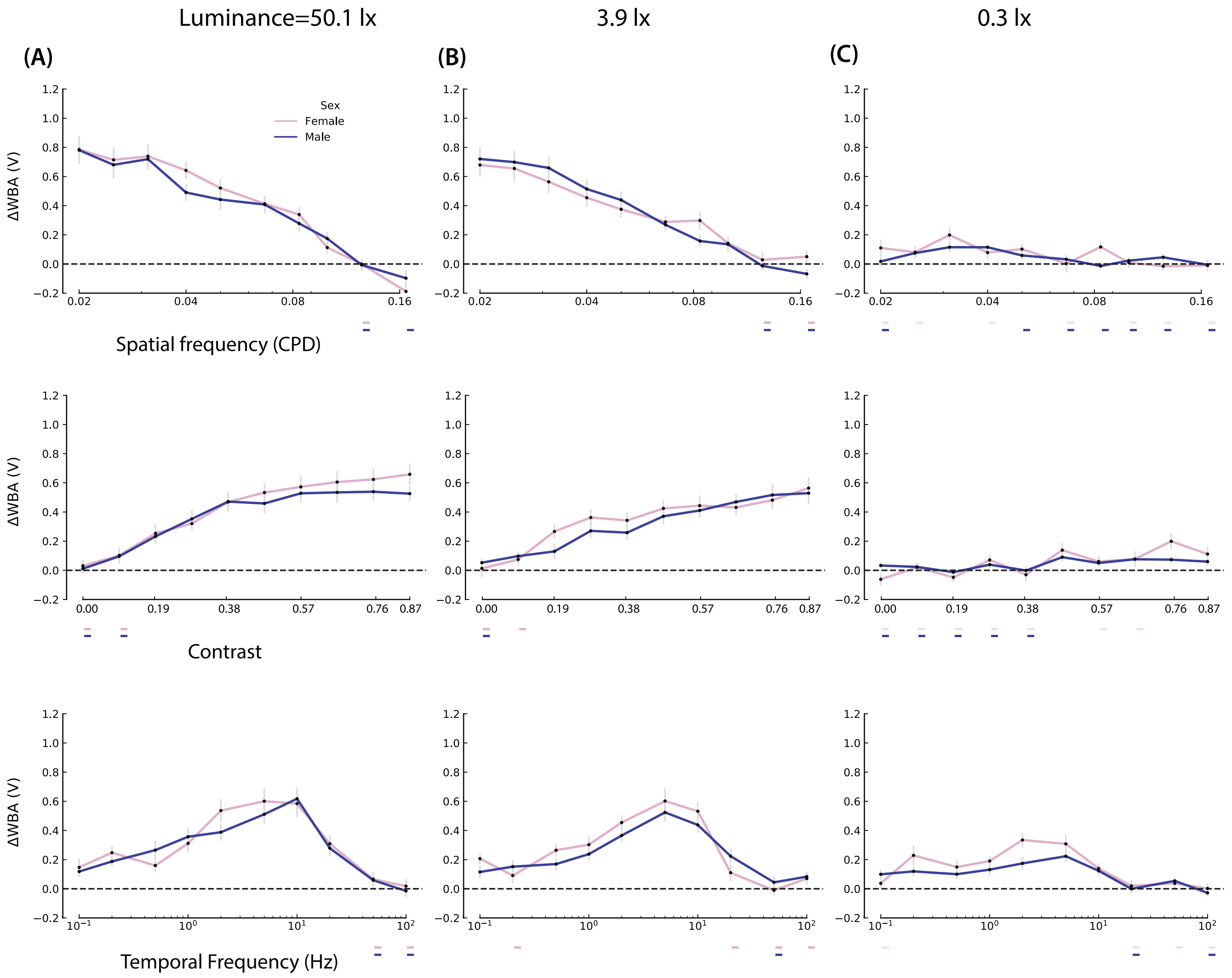

Fig. 5. A comparison of spatio-temporal visual abilities of male and female flies (n = 31 each) in the same range of eye area under different light intensities. Both males and females with an eye area of 0.14–0.19 mm^2 are compared here. Median eye area for both sexes is 0.17 mm^2, and mean ± SD are 0.1690 ± 0.013 and 0.166 ± 0.015 mm^2 for females and males respectively. Mean ± SE of ΔWBA is plotted for three light intensities: (A) 50.1 lx, (B) 3.9 lx, (C) 0.3 lx. Dashes below the x-axes indicate sample ΔWBA is not significantly different from 0 (one-sample *t*-test, $p \geq 0.05$).

small flies have a slightly higher contrast threshold than large flies (Fig. 3A). Although the energetic cost of neural summation and acute vision is not quantified, it is likely to affect the dim light adaptations in small flies if they were to use neural summation to a different extent than large flies. Such possible physiological differences between small and large flies remain to be tested.

5. Conclusions and future directions

This study has addressed the implications of eye size differences in fruit flies and visual capabilities under dim light conditions. We showed that both female and male fruit flies lose spatio-temporal visual abilities as light levels drop. Under dim light conditions, vision in small flies is more reduced than in large flies. Although such data are known in a few other insect species, the current study adds to the growing literature of a widely-studied model system, *Drosophila melanogaster*. Tethering restricts the flies in our study from using multisensory cues (such as air flow, odor, haltere balance) that could alter flight behaviors. Whether the differences in vision are reflected in their free flight behaviors under different light intensities is yet to be confirmed. It is possible that to cope with reduced visual abilities, small flies might avoid being active at low light intensities. If they are active, they may change their visual attention selectively towards areas with high perceivable information at a given light intensity (Palermo & Theobald, 2019). Alternatively, the precision and accuracy of their flight control behaviors, collision avoidance, and landing response may be affected under dim light conditions.

CRediT authorship contribution statement

Ravindra Palavalli-Nettimi: Conceptualization, Methodology, Visualization, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Jamie C. Theobald:** Conceptualization, Methodology, Visualization, Writing - review & editing, Supervision, Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank John P Currea for fruitful discussions and help towards the project; Nicholas Palermo, Carlos Ruiz, and Yash Sondhi for their feedback and support. This project is funded by National Science Foundation, grant number IOS-1750833.

References

Bachleitner, W., Kempinger, L., Wülbeck, C., Rieger, D., & Helfrich-Förster, C. (2007). Moonlight shifts the endogenous clock of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 3538–3543.

Baird, E., Fernandez, D. C., Wcislo, W. T., & Warrant, E. J. (2015). Flight control and landing precision in the nocturnal bee *Megalopta* is robust to large changes in light intensity. *Frontiers in Physiology, 6*, 1–7.

Cabrera, S., & Theobald, J. (2013). Flying fruit flies correct for visual sideslip depending on relative speed of forward optic flow. *Frontiers in Behavioral Neuroscience, 7*, 1–9.

Cheng, K. Y., Colbath, R. A., & Frye, M. A. (2019). Olfactory and neuromodulatory signals reverse visual object avoidance to approach in *Drosophila*. *Current Biology, 29*, 2058–2065.

Chiappe, M. E., Seelig, J. D., Reiser, M. B., & Jayaraman, V. (2010). Report walking modulates speed sensitivity in *Drosophila* motion vision. *Current Biology, 20*, 1470–1475.

Currea, J. P., Smith, J. L., & Theobald, J. C. (2018). Small fruit flies sacrifice temporal acuity to maintain contrast sensitivity. *Vision Research, 149*, 1–8.

de Haan, R., Lee, Y.-J., & Nordström, K. (2012). Octopaminergic modulation of contrast sensitivity. *Frontiers in Integrative Neuroscience, 6*, 1–10.

de Vries, H. L. (1943). The quantum character of light and its bearing upon threshold of vision, the differential sensitivity and visual acuity of the eye. *Physica, 10*, 553–564.

Doring, T. F., & Spaethe, J. (2012). Measurements of eye size and acuity in aphids (Hemiptera: Aphididae). *Entomol. Gen. 32*, 77–84.

Fischer, S., Müller, C. H. G., & Meyer-Rochow, V. B. (2011). How small can small be: the compound eye of the parasitoid wasp *Trichogramma evanescens* (Westwood, 1833) (Hymenoptera, Hexapoda), an insect of 0.3- to 0.4-mm total body size. *Visual Neuroscience, 28*, 295–308.

Fischer, S., Meyer-Rochow, V. B., & Müller, C. H. G. (2012). Challenging limits: Ultrastructure and size-related functional constraints of the compound eye of *Stigmella microtheriella* (Lepidoptera: Nepticulidae). *Journal of Morphology, 273*, 1064–1078.

Fischer, S., Meyer-Rochow, V. B., & Müller, C. H. G. (2014). Compound eye miniaturization in Lepidoptera: A comparative morphological analysis. *Acta Zoologica, 95*, 438–464.

Frederiksen, R., Wcislo, W. T., & Warrant, E. J. J. (2008). Visual reliability and information rate in the retina of a nocturnal bee. *Current Biology, 18*, 349–353.

Goosey, M. W., & Candy, D. J. (1980). The D-octopamine content of the haemolymph of the locust, *Schistocerca Americana gregaria* and its elevation during flight. *Insect Biochemistry, 10*, 393–397.

Gotz, K. G. (1987). Course-control, metabolism and wing interference during ultralong tethered flight in *Drosophila melanogaster*. *Journal of Experimental Biology, 128*, 35–46.

Götz, K. G. (1965). Die optischen Übertragungseigenschaften der Komplexaugen von *Drosophila*. *Kybernetik, 2*, 215–221.

Heisenberg, M., & Wolf, R. (1979). On the fine structure of yaw torque in visual flight orientation of *Drosophila melanogaster*. *Journal of Comparative Physiology, 130*, 113–130.

Honkanen, A., Immonen, E. V., Salmela, L., Heimonen, K., & Weckstrom, M. (2016). Insect photoreceptor adaptations to night vision. *Philosophical Transactions of the Royal Society of London. Series B, 372*, 20160077.

Jander, U., & Jander, R. (2002). Allometry and resolution of bee eyes (Apoidea). *Arthropod Structure, 30*, 179–193.

Kapustjanskij, A., Streinzer, M., Paulus, H. F., & Spaethe, J. (2007). Bigger is better: Implications of body size for flight ability under different light conditions and the evolution of alloethism in bumblebees. *Functional Ecology, 21*, 1130–1136.

Kelber, A., Warrant, E. J., Pfaff, M., Wallén, R., Theobald, J. C., Wcislo, W. T., & Raguso, R. A. (2006). Light intensity limits foraging activity in nocturnal and crepuscular bees. *Behavioral Ecology, 17*, 63–72.

Klaus, A., & Warrant, E. J. (2009). Optimum spatiotemporal receptive fields for vision in dim light. *Journal of Vision, 9*, 1–16.

Land, M. F. (1997). Visual acuity in insects. *Annual Review of Entomology, 42*, 147–177.

Lazopulo, S., Lazopulo, A., Baker, J. D., & Syed, S. (2019). Daytime colour preference in *Drosophila* depends on the circadian clock and TRP channels. *Nature, 574*, 108–111.

Lillywhite, P. G., & Laughlin, S. B. (1979). Transducer noise in photoreceptor. *Nature, 277*, 569–572.

Maimon, G. (2011). Modulation of visual physiology by behavioral state in monkeys, mice, and flies. *Current Opinion in Neurobiology, 21*, 559–564.

Maimon, G., Straw, A. D., & Dickinson, M. H. (2010). Active flight increases the gain of visual motion processing in *Drosophila*. *Nature Neuroscience, 13*, 393–399.

Majercak, J., Sidote, D., Hardin, P. E., Edery, I., Program, G., in, & Biochemistry (1999). How a circadian clock adapts to seasonal decreases in temperature and day length. *Neuron, 24*, 219–230.

Niven, J. E., & Laughlin, S. B. (2008). Energy limitation as a selective pressure on the evolution of sensory systems. *Journal of Experimental Biology, 211*, 1792–1804.

Palavalli-Nettimi, R., Ogawa, Y., Ryan, L. A., Hart, N. S., & Narendra, A. (2019). Miniaturisation reduces contrast sensitivity and spatial resolving power in ants. *Journal of Experimental Biology, 222*, jeb203018.

Palermo, N., & Theobald, J. (2019). Fruit flies increase attention to their frontal visual field during fast forward optic flow. *Biology Letters, 15*, 20180767.

Parthasarathy, K., & Willis, M. A. (2018). Parameters of motion vision in low light in the hawkmoth *manduca sexta*. *Journal of Experimental Biology, 221*, jeb173344.

Reber, T., Vähäkainu, A., Baird, E., Weckström, M., Warrant, E., & Dacke, M. (2015). Effect of light intensity on flight control and temporal properties of photoreceptors in bumblebees. *Journal of Experimental Biology, 218*, 1339–1346.

Rieger, D., Fraunholz, C., Popp, J., Bichler, D., Dittmann, R., & Helfrich-Förster, C. (2007). The fruit fly *Drosophila melanogaster* favors dim light and times its activity peaks to early dawn and late dusk. *Journal of Biological Rhythms, 22*, 387–399.

Rieke, F., & Baylor, D. A. (1996). Molecular origin of continuous dark noise in rod photoreceptors. *Biophysical Journal, 71*, 2553–2572.

Rose, A. (1942). The relative sensitivities of television pickup tubes, photographic film, and the human eye. *Proceedings of the IRE, 30*, 293–300.

Rutowski, R. L., Gislén, L., & Warrant, E. J. (2009). Visual acuity and sensitivity increase allometrically with body size in butterflies. *Arthropod Structure, 38*, 91–100.

Shingleton, A. W., Estep, C. M., Driscoll, M. V., & Dworkin, I. (2009). Many ways to be small: Different environmental regulators of size generate distinct scaling relationships in *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences, 276*, 2625–2633.

Snyder, A. W., Stavenga, D. G., & Laughlin, S. B. (1977). Spatial information capacity of compound eyes. *Journal of Comparative Physiology A, 116*, 183–207.

Spaethe, J., & Chittka, L. (2003). Interindividual variation of eye optics and single object resolution in bumblebees. *Journal of Experimental Biology, 206*, 3447–3453.

Spiewok, S., & Schmolz, E. (2006). Changes in temperature and light alter the flight speed of hornets (*Vespa crabro* L.). *Physiological and Biochemical Zoology, 79*, 188–193.

Sponberg, S., Dyhr, J. P., Hall, R. W., & Daniel, T. L. (2015). Luminance-dependent visual processing enables moth flight in low light. *Science (80-), 348*.

Stöckl, A. L., O'Carroll, D. C., & Warrant, E. J. (2016). Neural summation in the hawkmoth visual system extends the limits of vision in dim light. *Current Biology, 26*, 821–826.

Stöckl, A., Kihlström, K., Steven, C., & Sponberg, S. (2017a). Comparative system identification of flower tracking performance in three hawkmoth species reveals adaptations for dim light vision. *Philosophical Transactions of the Royal Society of London. Series B, 372*, 20160078.

Stöckl, A., O'Carroll, D. C., & Warrant, E. J. (2017b). Higher-order neural processing tunes motion neurons to visual ecology in three species of hawkmoths. *Proceedings of the Royal Society B: Biological Sciences, 284*, 20170880.

Streinzer, M., Huber, W., & Spaethe, J. (2016). Body size limits dim-light foraging activity in stingless bees (Apidae: Meliponini). *Journal of Comparative Physiology A, 202*, 643–655.

Tammero, L. F., Frye, M. A., & Dickinson, M. H. (2004). Spatial organization of visuomotor reflexes in *Drosophila*. *Journal of Experimental Biology, 207*, 113–122.

Taylor, G. J., Tichit, P., Schmidt, M. D., Bodey, A. J., Rau, C., & Baird, E. (2019). Bumblebee visual allometry results in locally improved resolution and globally improved sensitivity. *Elife, 8*, 1–32.

Theobald, J. (2017). Optic flow induces spatial filtering in fruit flies. *Current Biology, 27*, R212–R213.

Theobald, J. C., Greiner, B., Wcislo, W. T., & Warrant, E. J. (2006). Visual summation in night-flying sweat bees: A theoretical study. *Vision Research, 46*, 2298–2309.

Theobald, J. C., Coates, M. M., Wcislo, W. T., & Warrant, E. J. (2007). Flight performance in night-flying sweat bees suffers at low light levels. *Journal of Experimental Biology, 210*, 4034–4042.

Theobald, J. C., Warrant, E. J., & O'Carroll, D. C. (2010). Wide-field motion tuning in nocturnal hawkmoths. *Proceedings of the Royal Society B: Biological Sciences, 277*, 853–860.

Vanin, S., Bhutani, S., Montelli, S., Menegazzi, P., Green, E. W., Pegoraro, M., ... Kyriacou, C. P. (2012). Unexpected features of *Drosophila* circadian behavioural rhythms under natural conditions. *Nature, 484*, 371–375.

Warrant, E. J. (1999). Seeing better at night: Life style, eye design and the optimum strategy of spatial and temporal summation. *Vision Research, 39*, 1611–1630.

Warrant, E. J. (2008a). Nocturnal vision. In T. Albright, & R. H. Masland (Eds.). *The senses: A comprehensive reference* (pp. 53–86). Oxford; Academic Press.

Warrant, E. J. (2008b). Seeing in the dark: Vision and visual behaviour in nocturnal bees and wasps. *Journal of Experimental Biology, 211*, 1737–1746.

Warrant, E. J. (2017). The remarkable visual capacities of nocturnal insects: Vision at the limits with small eyes and tiny brains. *Philosophical Transactions of the Royal Society of London. Series B, 372*, 20160063.

Warrant, E., & Dacke, M. (2011). Vision and visual navigation in nocturnal insects. *Annual Review of Entomology, 56*, 239–254.