Vision Research 149 (2018) 1–8

Contents lists available at ScienceDirect

Vision Research

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

Small fruit flies sacrifice temporal acuity to maintain contrast sensitivity

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ARTICLE INFO

Keywords:
Acuity
Sensitivity
Development
Plasticity
Drosophila
Fruit fly

ABSTRACT

Holometabolous insects, like fruit flies, grow primarily during larval development. Scarce larval feeding is common in nature and generates smaller adults. Despite the importance of vision to flies, eye size scales proportionately with body size, and smaller eyes confer poorer vision due to smaller optics. Variable larval feeding, therefore, causes within-species differences in visual processing, which have gone largely unnoticed due to ad libitum feeding in the lab that results in generally large adults. Do smaller eyes have smaller ommatidial lenses, reducing sensitivity, or broader inter-ommatidial angles, reducing acuity? And to what extent might neural processes adapt to these optical challenges with temporal and spatial summation? To understand this in the fruit fly, we generated a distribution of body lengths (1.67 − 2.34 mm; n = 24) and eye lengths (0.33 − 0.44 mm; n = 24), resembling the distribution of wild-caught flies, by removing larvae from food during their third instar. We find smaller eyes (0.19 vs.0.07 mm^2) have substantially fewer (978 vs. 540, n = 45) and smaller ommatidia (222 vs. 121 μm^2;n = 45) separated by slightly wider inter-ommatidial angles (4.5 vs.5.5°; n = 34). This corresponds to a greater loss in contrast sensitivity (< 50%) than spatial acuity (< 20%). Using a flight arena and psychophysics paradigm, we find that smaller flies lose little spatial acuity (0.126 vs. 0.118CPD; n = 45), and recover contrast sensitivity (2.22 for both; n = 65) by sacrificing temporal acuity (26.3 vs. 10.8Hz; n = 112) at the neural level. Therefore, smaller flies sacrifice contrast sensitivity to maintain spatial acuity optically, but recover contrast sensitivity, almost completely, by sacrificing temporal acuity neurally.

1. Introduction

In general, larger animals have eyes that are larger in absolute terms but smaller relative to body size (Hughes, 1977; Rensch, 1948; Stevenson, Hill, & Bryant, 1995). Because optical quality is limited by the eyes' absolute and not relative size (Land & Nilsson, 2012), progressively smaller animals face an increasingly difficult optical challenge. Substantial comparative work has demonstrated evolutionary adaptations in the optics and neural processing of visual systems to cope with small apertures (Hughes, 1977; Krapp, 2000; Land & Nilsson, 2012; Theobald, Warrant, & O'Carroll, 2010). However, though body and eye size can also vary substantially within species (Shingleton, Estep, Driscoll, & Dworkin, 2009; Shingleton, Frankino, Flatt, Nijhout, & Emlen, 2007), little is known about what developmental adaptations smaller-eyed conspecifics employ.

The fruit fly, with two neural superposition compound eyes, each about 0.15 mm^2 in area, exemplifies this small-eyed developmental challenge. Limited food availability during the fruit fly's late larval stages, a common condition in nature, results in smaller adults with smaller eyes (Callier & Nijhout, 2013; Shingleton et al., 2007, 2009). Each eye is an approximate hemisphere composed of about 800 nearly identical ommatidia, each containing 1 lens that focuses light upon 8 photoreceptors (Ready, Hanson, & Benzer, 1976). This geometric arrangement dictates that smaller eyes must confer poorer vision due to a decrease in the size of each ommatidial lens, an increase in the angle between ommatidia, or some combination of both. Neural summation processes might compensate for some of this loss, but only at the expense of some form of acuity (Warrant, 1999). Although small adults are common in nature where larval food availability and other environmental factors are highly variable, fly vision is mostly studied with uniformly large, lab-reared adults, and how small adults cope with small optics is unknown. Here we measure the sacrifices made by smaller flies at the optical level, and the summation processes they employ at later stages.

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

The size of a holometabolous insect in general, and a fruit fly in

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https://doi.org/10.1016/j.visres.2018.05.007
Received 23 January 2018; Received in revised form 3 May 2018; Accepted 23 May 2018

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

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

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

A general principle of vision is that spatial acuity, or visual sharpness, and contrast sensitivity, or the ability to discriminate luminance levels, trade off (Land, 1997; Land & Nilsson, 2012; Theobald et al., 2010). Contrast sensitivity is determined by the amount of light absorbed by each photoreceptor, which is limited in the fruit fly by ommatidial diameter (Fig. 1C, labeled D). The contrast sensitivity, S, of an eye to an extended light source is calculated by: $S = \left(\frac{\pi}{4}\right)^2 D^2 \left(\frac{d}{f}\right)^2 \left(\frac{kl}{2.3+kl}\right)$, where D is ommatidial diameter (μm), f is ommatidial focal length (μm), and d, l, and k the diameter (μm), length (μm), and absorption coefficient (photons μm^{-1}) of each photoreceptor rhabdomere (Warrant & Nilsson, 1998). Spatial acuity is inversely determined by the angle between adjacent ommatidia, the inter-ommatidial angle (Fig. 1C, labeled Δφ; Land & Nilsson, 2012). The highest discernible spatial frequency, ν_{max}, of a hexagonal lattice like the fruit fly's eye is given by: $\nu_{max} = \frac{1}{\sqrt{3}\Delta\phi}$. The fundamental acuity-sensitivity tradeoff is demonstrated by the eye's geometry, such that decreasing Δφ, which increases spatial acuity, necessarily decreases D, which decreases contrast sensitivity, and vice versa (Land & Nilsson, 2012). Likewise, reducing eye size necessarily decreases D, increases Δφ, or some combination of both. As a result, smaller flies, who have smaller eyes, must sacrifice at least one of the two visual properties, acuity or sensitivity, and the overall image quality.

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

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

Low light absorption due to smaller ommatidia presents the same problem as that faced by all animals viewing images in dim light: how to resolve an accurate image with fewer photons? Both vertebrate and invertebrate visual systems improve the visible range of ambient light intensities by increasing the receptive field of visual interneurons, via

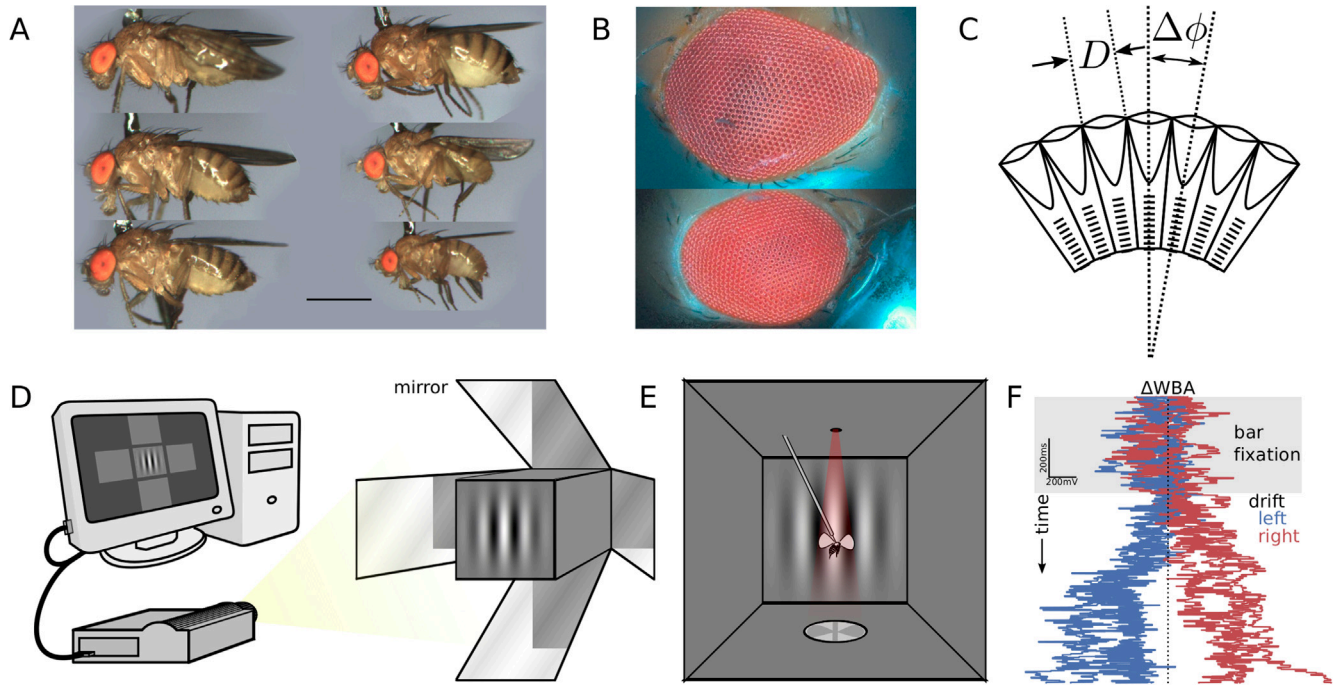

Fig. 1. (A) Lab-reared adults that were abundantly fed as larvae (left) are generally larger than those who had limited larval food availability (right). (B) Eyes are proportionate to the size of the overall body. (C) Ommatidial diameter, labeled D, limits contrast sensitivity, while inter-ommatidial angle, labeled Δφ, inversely limits spatial acuity. Because ommatidial diameter is directly proportional to inter-ommatidial angle, the two visual properties of sensitivity and acuity trade off. (D) A computer generates visual stimuli projected onto 5 surfaces of the flight arena via 4 mirrors. (E) In the flight arena, each of the fly's wingbeats are captured by an infrared light and two receivers. (F) The difference in wing beat amplitudes, ΔWBA, signals the fly's steering effort.

spatial summation, or increasing the integration time of phototransduction, via temporal summation (Warrant, 1999; Warrant & Nilsson, 2006). However, spatial and temporal summation strategies improve contrast sensitivity only by sacrificing spatial and temporal acuity. Spatial summation increases the functional inter-receptor angle, improving contrast sensitivity while sacrificing spatial acuity according to the acuity-sensitivity tradeoff discussed in Section 1.2. The fruit fly has demonstrated spatial summation in response to optic flow, quickly reducing their peripheral spatial acuity from about 0.1 CPD to 0.07 CPD to improve contrast sensitivity in regions most affected by motion blur (Theobald, 2017). Similarly, temporal summation allows for greater photon capture but reduces temporal acuity, or the fastest discernible change in luminance. During dark adaptation, fruit fly photoreceptors increase integration time and consequently restrict themselves to a lower bandwidth of temporal frequencies, reducing their temporal acuity (Juusola & Hardie, 2001). Intracellular measurements show that fruit fly temporal acuity decreases approximately from 30 Hz to 10 Hz over a 4 log unit reduction in ambient light (Juusola & Hardie, 2001; depending on methodology, higher values have been measured for temporal acuity; for example, see Cosens & Spatz, 1978). Reductions in temporal acuity can be detrimental for quickly moving animals and animals' use of spatial versus temporal summation corresponds greatly to their visual ecology (Krapp, 2000; Theobald et al., 2010; Warrant, 1999).

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

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

2. Materials and methods

2.1. Subjects

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

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

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

2.2. Optical parameters

Body length, ommatidial count, eye area, and ommatidial area were measured using a digital recording microscope (Zeiss Axio Scope.A1) and a custom python script using human input. Body length was measured as the shortest distance from the tip of the head to the end of the abdomen. For eye measurements, multiple images (~20) were taken per fly from one angle, at fixed intervals of focus depth. A custom python script generated a single image composite of the stack of photos, using the Sobel operator to choose the pixel of highest focus from the stack of images. The final focus stack displays the entire eye in focus, allowing us to count the ommatidia and take direct measurements on a computer display. The projected eye area was calculated by fitting an ellipse to the contour of the eye. Ommatidial area was approximated by averaging the length of 81 ommatidia near the center of the eye and approximating the lens as a circle.

Inter-ommatidial angle was measured for each fly by using a precise goniometer under a digital recording microscope. Using the pseudopupil as a guide, the eye was positioned so that (1) the microscope stared directly down an ommatidium and (2) the center of rotation of the eye matched that of the goniometer. The initial angle on the goniometer was recorded and the eye was pitched by 20 one-degree intervals. The distance covered by 6 ommatidia was measured at each interval and then averaged. This approximates the 1° arc of the eye, L. Notice that the proportion of the inter-ommatidial angle to one degree is equal to the proportion of the ommatidial diameter, D, to the one degree arc, L, allowing us to approximate the inter-ommatidial angle as, $\Delta\varphi = D/L$, in degrees. (Land, 1997).

2.3. Allometry

Allometric scaling between physical traits usually follow a power law, $Y = aX^b$, where Y and X are traits and a and b are constants (Shingleton et al., 2007; Voje, Hansen, Egset, Bolstad, & Pélabon, 2014). b is known as the allometric constant and represents the rate of growth of Y in comparison to X. For $a > 0$, $b = 1$ implies isometry or 1:1 scaling between X and Y; $b < 1$ implies hypoallometry, so that as X increases, Y increases at a decreasing rate; $b > 1$ implies hyperallometry, so that as X increases, Y increases at an increasing rate. These same interpretations apply for $b < 0$, except they imply inverse allometries.

2.4. Flight arena

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

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

2.5. Stimulus

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

2.6. Moving sinusoidal gratings

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

3. Results

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

One-way analysis of variance tests demonstrate that at least one of the four conditions is significantly different in both body ($F(144, 3) = 28.60$, $p < 0.01$) and eye size ($F(144, 3) = 71.84$, $p < 0.01$). Post hoc analysis using Tukey's honestly significant difference test is used to assess pairwise differences (see Fig. 2). Compared to wild flies, lab flies have an approximately 13% longer body (95%C.I. = [0.16, 0.48] mm, $p < 0.01$) and 15% larger eyes (95%C.I. = [0.05, 0.10] mm, $p < 0.01$). Compared to lab flies, lab-restricted flies have an approximately 19% smaller body (95%C.I. = [0.26, 0.64] mm, $p < 0.01$) and 24% smaller eyes (95%C.I. = [0.09, 0.15] mm, $p < 0.01$). Lab-restricted flies are the same size as wild flies (95%C.I. = [−0.31, 0.06] mm, ns) and have eyes that are about 6% smaller than wild flies (95%C.I. = [−0.07, −0.01] mm, $p < 0.01$). Finally, wild-fed flies are the same size (95%C.I. = [−0.21, 0.12] mm, ns) and have the same eye size (95%C.I. = [−0.04, 0.01] mm, ns) as lab flies.

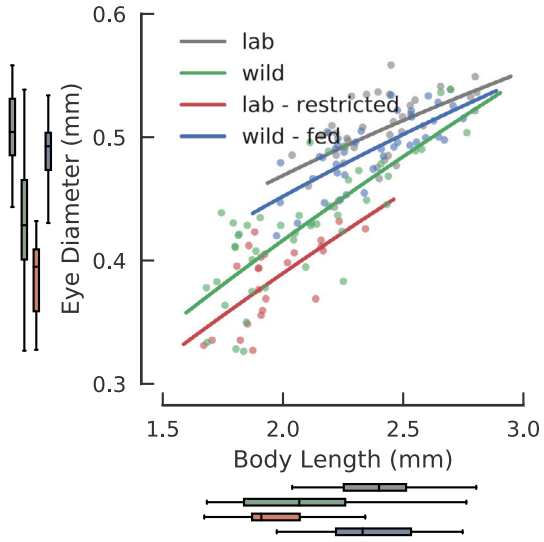

Fig. 2. Body and eye sizes differ between 4 rearing conditions. In gray are lab-reared flies exposed to abundant larval feeding. In green are wild caught flies. In red are lab-reared flies exposed to restricted larval feeding. In blue are the progeny of wild-caught flies exposed to abundant larval feeding. Box plots on each axis show the range, inter-quartile range, and median for each group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Despite some differences in absolute sizes, the scaling relations of eye length and body length did not differ substantially between groups. Each scaling relation fits well into the allometric model (lab: $p < 0.01$, $R^2 = 0.40$; wild: $p < 0.01$, $R^2 = 0.60$; lab-restricted: $p < 0.01$, $R^2 = 0.44$; wild-fed: $p < 0.01$, $R^2 = 0.56$). For each condition, eye length scales hypoallometrically with respect to body length (lab: $b = 0.41$, 95%C.I. = [0.23, 0.59]; wild: $b = 0.68$, 95%C.I. = [0.51, 0.85]; lab-restricted: $b = 0.69$, 95%C.I. = [0.35, 1.03]; wild-fed: $b = 0.47$, 95%C.I. = [0.33, 0.61]). While the allometric constants do not differ greatly (their confidence intervals overlap), they are more similar between larval feeding conditions than related individuals: the constant for lab flies, $b = 0.41$, is closer to that of wild-fed flies, $b = 0.47$, than lab-restricted flies, $b = 0.69$; the constant for wild flies, $b = 0.68$, is closer to that of lab-restricted flies, $b = 0.69$, than wild-fed flies, $b = 0.47$. Larval feeding, therefore, is a strong predictor of adult eye to body scaling relations and restricting larval feeding of lab bred flies generates a range of adult body and eye sizes more similar to those in the wild.

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

The scaling of both ommatidial count and ommatidial area with eye area (Fig. 3A and B) each fit well into the allometric model ($p < 0.001$ for both and $R^2 = 0.951$ and $R^2 = 0.954$, respectively). Ommatidial count scales positively and hypoallometrically with respect to eye area ($b = 0.58$, 95%C.I. = [0.538, 0.629]; $a = 2502$, 95%C.I. = [2310, 2710]), meaning that smaller eyes have fewer ommatidia but have a higher ratio of ommatidial count to eye area, or ommatidial density. Ommatidial count ranged from 540 ommatidia in the smallest eye to 978 ommatidia in the largest, an increase of 45%. Inversely, ommatidial

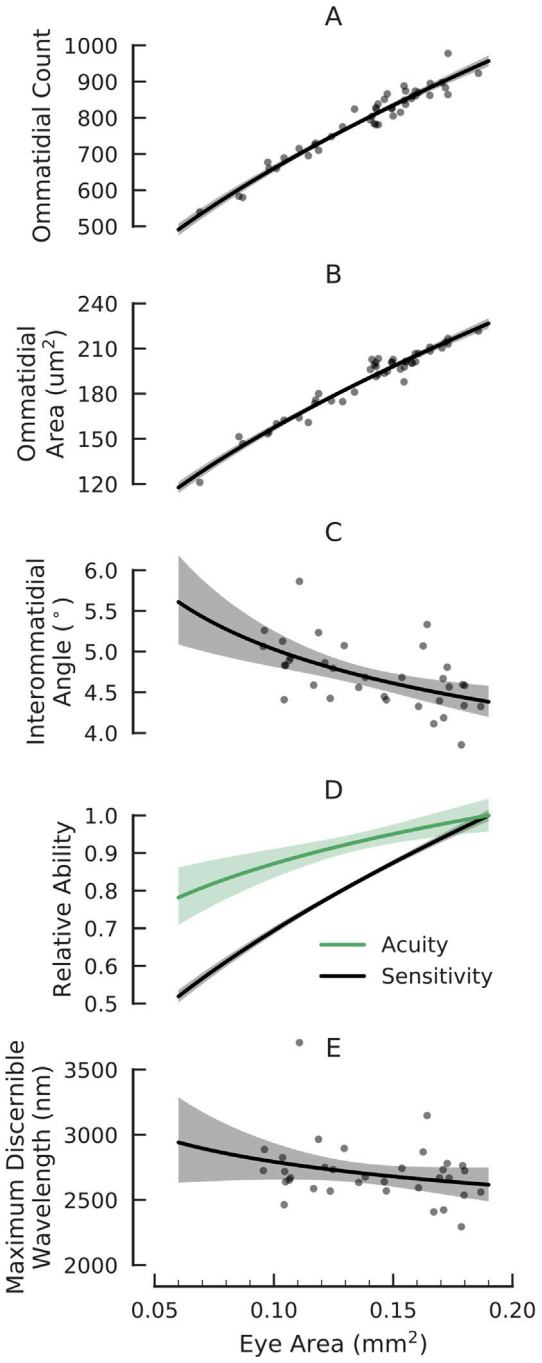

Fig. 3. Smaller eyes have as much as 45% fewer (A) and 45% smaller (B) ommatidia separated by as much as 20% wider inter-ommatidial angles (C). The scaling relations of B and C affect contrast sensitivity and spatial acuity differently (D), such that smaller eyes sacrifice contrast sensitivity (black) as much as 30% more than spatial acuity (green). The minimum visible wavelength of light due to diffraction is not significantly affected by smaller eye size (E) due to the coordinated decrease of ommatidial area and increase of inter-ommatidial angle. *Note:* Filled areas represent 95% confidence bands of the mean based on the allometric (log-transformed) model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

densities ranged from 4970 ommatidia mm^{-2} in the largest eye to 7833 ommatidia mm^{-2} in the smallest eye, an increase of 37%.

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

The scaling of inter-ommatidial angle with eye area (Fig. 3C) fits well into the allometric model ($p < 0.001$ and $R^2 = 0.31$). Inter-ommatidial angle scales inversely and hypoallometrically with eye size ($b = -0.21$, 95% C.I. = [−0.329, −0.099]; $a = -3.07$, 95%C.I. = [2.445, 3.861]), meaning that smaller eyes have disproportionately wider inter-ommatidial angles.

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

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

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

3.4. Behavior

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

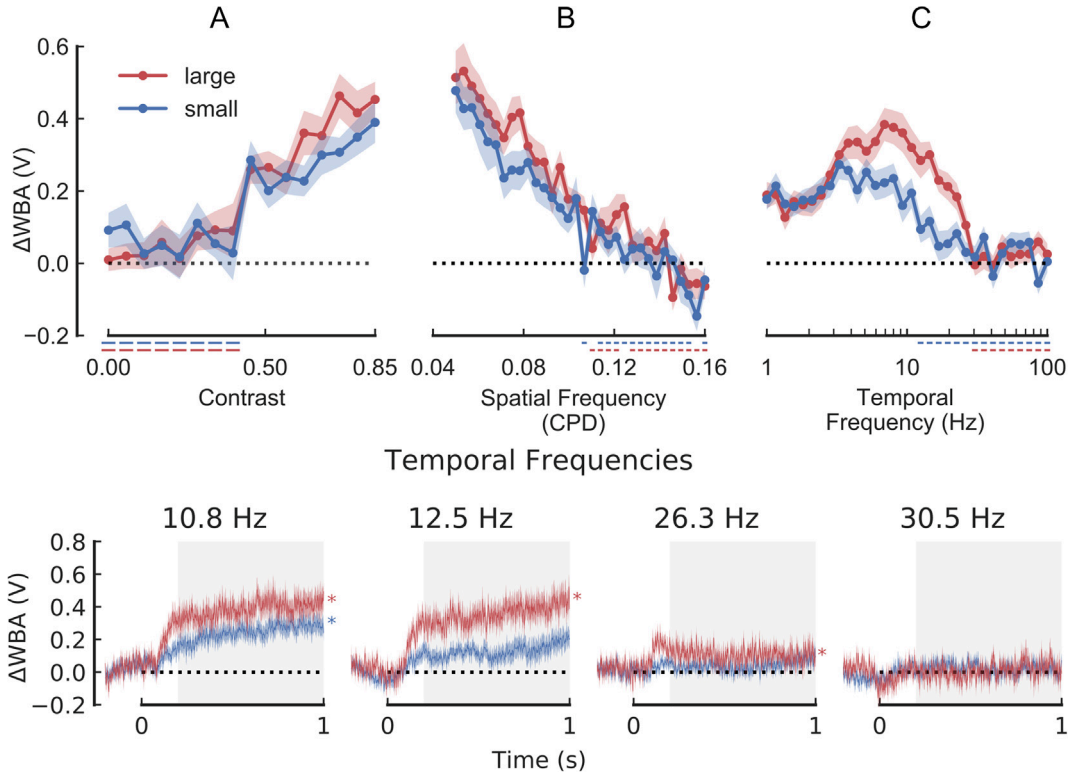

Fig. 4. Behavioral measurements of contrast sensitivity (A), spatial acuity (B), and temporal acuity (C) for large (red) and small (blue) flies. ΔWBA is the difference in wingbeat amplitude between the left and right wings, normalized here so that a positive response is in the direction of the sine grating. Top: plots show mean ΔWBA responses ± SEM taken over the last 800 ms of each trial. Colored dashes below the horizontal axis signify when the mean is not significantly different from 0 using a one sample *t*-test at P = 0.01. Bottom: time series of the mean ΔWBA response ± SEM for large and small flies to four select temporal frequencies demonstrate how both small and large flies can respond reliably up to 10.8 Hz, but only large flies respond reliably to 26.3 Hz. Temporal frequency refers to the contrast frequency of the sinusoidal pattern moving at constant speed and not, for instance, the refresh rate of the projector. Asterisks signify when the mean during the last 800 ms is significantly different from 0 using a one sample *t*-test at P = 0.01. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Contrast sensitivity, measured as the lowest discernible contrast, was not significantly affected by eye size (Fig. 4A). $N = 65$ flies were tested and split into two bins along their median eye area of 0.11 mm^2. The small group had a mean eye area of 0.09 mm^2 and the large group had a mean eye area of 0.14 mm^2. Despite these differences in eye size, corresponding to substantial differences in ommatidial size, both demonstrated a lowest discernible contrast of 0.45 (small: $t(31) = 5.29$, $p < 0.01$; large: $t(32) = 6.58$, $p < 0.01$). This corresponds to a contrast sensitivity (1/threshold Michelson contrast) of 2.22.

Based on the allometric scaling of eye area and ommatidial area measured in Section 3.1, the two eye size groups had an estimated ommatidial area of 149.3 μm^2 for the small eyes and 191.5 μm^2 for the large eyes. This should correspond to a 22% sacrifice in contrast sensitivity for the small eyes. The absence of any significant behavioral sacrifice in contrast sensitivity suggests that smaller-eyed flies are using spatial or temporal summation to compensate, with implications for their spatial or temporal acuity.

Spatial acuity, measured as the highest discernible spatial frequency, improved moderately with eye size (Fig. 4B). $N = 45$ flies were tested and split into two bins along their median eye area of 0.14 mm^2. The small group had a mean eye area of 0.12 mm^2 and the large group had a mean eye area of 0.16 mm^2. These differences in eye size, corresponding to moderate differences in inter-ommatidial angle, resulted in the larger eyes' ability to discern spatial frequencies up to about 0.125 CPD ($t(22) = 4.41$, $p < 0.01$) while the smaller eyes could discern only up to 0.11 CPD ($t(21) = 2.91$, $p < 0.01$). This represents a 12% decrease in spatial acuity for smaller eyes.

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

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

4. Discussion

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

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

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

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

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

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

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

4.3. Loss of temporal acuity occurs in dark-adapted and dark-reared photoreceptors

When adapting to dimmer environments, the photoreceptors of normally sized fruit flies, and many other insects, increase their membrane conductance, hyperpolarizing the cell and increasing the cell's time constant (Juusola & Hardie, 2001). This has the effect of a low pass filter with a cutoff frequency that corresponds to ambient light levels (Juusola & Hardie, 2001). Decreased photon capture due to a smaller lens is not fundamentally different than that due to dim ambient light levels, so small flies might use the same mechanism for dark adaptation to recover contrast sensitivity lost by their smaller optics.

While dark adaptation results in lower temporal acuity of the photoreceptor (Juusola & Hardie, 2001), it is a minor decrease compared to what we found in our behavioral experiments. Small eyes had an estimated ommatidial area of about 160 μm^2, and large eyes had an estimated ommatidial area of about 205 μm^2, suggesting a decrease of about 25% of the light available to the smaller eyes' photoreceptors. This corresponded to a decrease in temporal acuity from about 26 Hz for the large eyes to about 13 Hz for the small eyes. For comparison, an average photoreceptor demonstrates a temporal frequency cutoff of about 25 Hz in ambient light of about 3×10^6 photons per second (Juusola & Hardie, 2001). After a 90% decrease in ambient light, the cutoff decreases to around 23 Hz, only 3 Hz less (Juusola & Hardie, 2001). A decrease of 10 Hz, like we found in smaller flies at the behavioral level, occurs in normal photoreceptors only after a decrease of about 99.7% in ambient light (Juusola & Hardie, 2001). However, the differences at the behavioral level may stem from smaller differences in the retina or may involve an entirely different mechanism. Future research should use intracellular electrophysiology to determine the mechanism underlying this trade-off and how it might relate to dark adaptation.

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

4.4. Loss of temporal acuity has ecological implications

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

Temporal acuity may differ between the lateral and frontal regions of the eye. During forward motion, images in the periphery move quicker than those in front, so temporal summation would increase motion blur in the lateral regions of the eye more than in the front. Small eyes may have higher temporal acuity in the lateral regions of their eye to minimize the motion blur differences between small and large eyes. What, then, would be their peripheral contrast sensitivity? This remains unknown because we displayed gratings in the front 90

degrees of the flight arena. Interestingly, fruit flies can increase spatial summation in the periphery in response to optic flow, which attenuates the effect of motion blur (Theobald, 2017). Further research will investigate temporal processing in different regions of small versus large eyes and how this might interact with optic flow-induced spatial filtering.

4.5. Conclusion

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

Acknowledgments

We thank Nicolas Palermo and Carlos Ruiz for comments on the manuscript. This work was supported by the National Institutes of Health/National Institutes of General Medical Sciences, grant number R25 GM061347 and the National Science Foundation, grant number IOS-1750833. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Science Foundation.

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