STRUCTURE AND FUNCTION OF THE COMPOUND EYE IN PHOTINUS CAROLINUS (LAMPYRIDAE): REGIONAL VARIATION AND VISUAL PERFORMANCE

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INTRODUCTION

Fireflies use visual signals to communicate information about their gender and species [1,6-7]. Typically, a male produces a species-specific flash while flying and looking for the flash response of a conspecific female. Females detect and recognize the flashes of conspecific males and respond with their own flash pattern. Males orient towards the flashing female and land in her vicinity. Sexual dimorphism of the size of the firefly compound eye has been described previously [3,10] and confirmed in preliminary studies with male and female *Photinus carolinus* (Birdsey and Moiseff, personal communication). The ommatidium is the basic unit of the compound eye; each ommatidium is associated with a surface lens, or facet [3,10]. The number, size, spatial distribution of the facets, eye radii, receptor widths, and focal lengths determine the eye's visual performance. Accordingly, variations in the structure of the insect compound eye may reflect specializations that relate to the functional capabilities of an insect, see for example: [2,8].

Visual performance in the compound eye can be understood in the terms of optical resolution and sensitivity. The optical properties of the structure differences were investigated by modeling the physiological optics through measured and calculated data from photomicrographs and anatomical microscopic sections. Based on both methods, there were dimorphic and regional variations observed in optical aspects such as facet's size and number, eye radii, receptors widths, local focal lengths, resolution and sensitivity which reflect their visual performance and behaviour. The measurements obtained were put into theoretical framework to relate surface dimensions to the optical properties [3,5].

Both resolution and sensitivity are important to the processing and the quality of the image seen by the brain. Longer focal length (larger eye) is better for obtaining small resolvable angle and high sampling frequency (νs) = f/2d = 1/ (2$\Delta\phi$), where f is the focal length, d is receptor separation, and $\Delta\phi$ is the inter-receptor angle [5]. Wide aperture (facet size, D) is needed for two reasons; for enough light gathering and to reduce the diffraction and therefore higher cut-off frequency (νco) = D/λ, where D is the facet diameter and λ is the wavelength. The optimal eye would be one that resolves well in wide range of light conditions. Fireflies which are active at night have superposition eyes which are more sensitive to light than apposition eyes. We hypothesize that the gross structural differences between the male and female *P. carolinus* eyes reflect specializations that support different functional capabilities in males and females.

METHODS

Geometrical distortion is inherent in any imaging system where a spherical surface is projected onto a flat, 2-dimensional *image plane*. We designed a miniature stage goniometer system to make accurate and repeatable angular rotations of a hemispherical compound eye in polar motion around a great-circle arcs (Fig. 1), where the term great-circle arc refers to a line on the surface of a sphere that is formed from the intersection of a plane passing through the center of the sphere (for details see[9]).

The areas of the middle approximate 40 facets (Fig. 1-d) were measured using ImageJ [11] and calculated using Excel/SigmaPlot and plotted in Matlab (MathWorks). Results for each great circle arc scan were processed and a contour map (Fig. 2) of the averages was plotted in polar coordinates corresponding to the angles of rotation of the mounting pin and the angle along the great circle arc. Differences within regions of the eye were compared using a paired t-test. Differences between regions of the eyes were compared using an unpaired t-test.

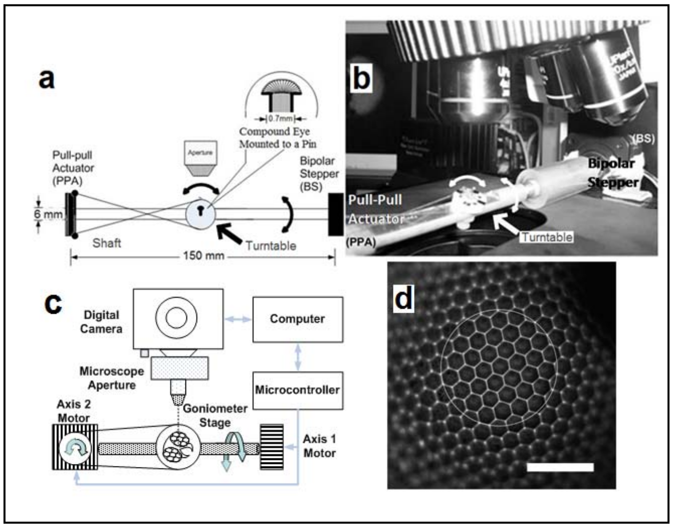

Figure 1: Goniometer. **a** Schematic design of the stage goniometer. The eye or head was mounted to a pin that was centered on a rotating turntable (straight arrow). The turntable assembly pivoted around the axis of the shaft. **b** Photograph of the goniometer in use on the stage of a light microscope. The sample was illuminated from above using a fiber optic illuminator (not shown). **c** System block diagram of the computerized stage goniometer for eye mapping **d** Image of surface of firefly compound eye. Facet measurements were done off-line using images obtained with a 20x objective. Average facet area was calculated from approximately 40 facets in the center of each image (circle). The inability to bring the entire image field into focus was the result of the curvature of the surface. Scale bar = 100 μm. (Figure modified from [9])

The results reported were obtained from two male and two female *Photinus carolinus* fireflies that were collected in the Great Smoky Mountains National Park, USA (Permit No. GRSM.2005.SCI.0035) and air-dried.

RESULTS

The eyes of the male and female fireflies were dimorphic (Table 1). In *P. carolinus*, the diameter of the male's eyes was 32% larger than the diameter of the female's eyes.

Table 1: Comparison of morphological features of the compound eye in both sexes for *P. carolinus* firefly. Abbr. R = eye radius, ϕ = angle around the centroid, α = angle behind the center of the eye, X = the segment behind the hemisphere, SA = surface area of the eye, FA = average facet area, and N = calculated number of facets per eye.

Surface Feature	♂ ***Photinus carolinus***	♀ ***Photinus carolinus***
R (μm)	583	439
ϕ (°)	220.5	198.5
α (°)	20.25	9.33
X (μm)	200	72
SA (μm^2)	2.87 x 10^6	1.41 x 10^6
FA (μm^2)	497	432
N	5771	3263

In addition to the larger diameter, the male eye extended 220.5° around the sphere, compared to only 198.5° for the female. The average facet area in *P. carolinus* was slightly larger 15% in males than females. Although the male eye has larger facet areas, when combined with its substantially larger eye diameter this results in males' eyes having many more facets than females' eyes.

In the example shown, average facet area ranged from approximately 410 μm^2 at the dorsal-lateral region to approximately 550 μm^2 in the central region.

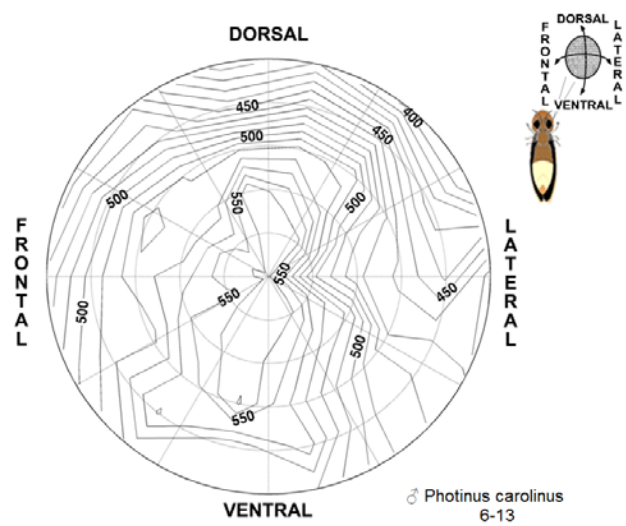

Figure 2: Contour polar plot of average facet area of male *Photinus carolinus* compound eye. Facet area is measured in μm². Upper right: Sketch of a *ventral* view of the firefly illustrating the nomenclature for eye

region location. The *frontal* region of the eye was defined as the region closest to the base of the antenna, see [9].

Seven great circle arc scans, each containing up to 12 images, were sufficient to image the eye surface resulting in as many as 84 images. Average facet area was calculated for each of these images and the results displayed in polar coordinates as an azimuthal projection (Fig. 2). We consistently observed that the facets having the largest area were located in the center of the polar plot, corresponding to the *central* region of the eye. The smallest facet areas (approximately 410 μm^2) were located on the most *dorsal* and *dorsal-lateral* edges of the eye (Fig. 2).

To summarize the relationship between facet size and position on the eye, we selected five regions of each eye corresponding to the *central, dorsal, ventral, frontal and lateral* aspects . The *frontal* region was defined as the portion of the eye closest to the base of the ipsilateral antenna (Fig. 2, upper right). In each of these regions, the areas of approximate 40 facets from a representative area (e.g. Fig. 1-d) were averaged for each sex. The regional averages were displayed on the figure to represent schematically the relative positions of the 5 selected eye regions (Fig. 3). In our *P. carolinus* female samples, facet area varied from 378 ±11.27 μm^2 in the *dorsal* region to 458 ±7.45 μm^2 in the ventral region ($p<<0.01$, unpaired t-test). Regional variations were also seen in males, for example our male *P. carolinus* had an average facet area of 458 ±18.79 μm^2 in the *dorsal* region compared to 527 ±8.81 μm^2 in the ventral region ($p << 0.01$, unpaired t-test).

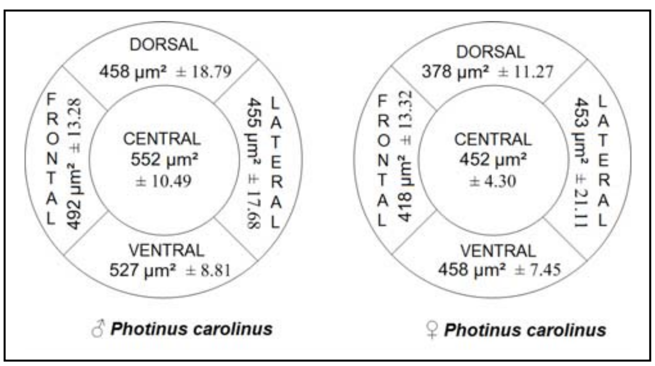

Figure 3: Schematic diagram summarizing the average facet area of male and female in *P. carolinus* firefly. Data obtained from surface imaging using goniometer.

Visual performance data related to resolution and sensitivity were extracted from measurements which were taken at three regions of the cross sections of the *P. carolinus* eye (Table 2); Frontal, Central, and Lateral, correspondingly, based on the anatomical microscopic cross sections of both sexes.

Table 2: Regional variations in the optical properties across the compound eyes in both sexes in *Photinus carolinus*: Abbr. D is the facet size, d is the receptor separation, f is focal length, R is eye radius, S is the Sensitivity which is = 0.62 (D^2) (Δρ^2), where Δρ is the acceptance angle = √ {[(λ/D)^2] + [(d/f)^2]}, λ is wavelength = 0.5µm, (νs) is sampling frequency; νco is cut-off frequency; and $\Delta\phi$ is interommatidial angle in Radian. Method: Surface Imaging method (SI) = measurement and calculation from images acquired through goniometer and light microscope, Anatomical method (A) = measurement and calculation were done from 7.5 µm thick sections, and Anatomical/Geometrical method (AG) = calculation based on geometrical centroid.

	Frontal		**Central**		**Lateral**		**Method**
	♀	♂	♀	♂	♀	♂	
D (µm)	22	24	23	25	23	23	SI
d (µm)	15	16	15	16	15	16	A
f (µm)	218	385	229	385	258	385	A
R (µm)	439	583	439	583	439	583	SI
	407	594	407	594	407	594	AG
$\Delta\phi$	0.05	0.04	0.05	0.04	0.05	0.04	SI
νco	44	47.7	45.7	50.5	45.7	45.8	SI
νs	10	12	9.6	11.6	9.6	12.8	SI
	7.3	12	7.6	12	8.6	12	A
Δρ	0.08	0.05	0.07	0.05	0.08	0.05	A
S (µm²)	177	76	174	84	140	72	A

The optical properties of the compound eye in *P. carolinus* showed sexual dimorphism and confirmed regional variations in most of their optical aspects such as facet's size and number, eye radii, receptors widths, local focal lengths, resolution and sensitivity. Such vvariation may be related to behavioural and functional capabilities in *P. carolinus*.

DISCUSSION

The compound eye allows a firefly to sense and interact with its visual environment. The number, size, distribution, and orientation of the lens-like facets comprising the eye are fundamental contributors to the eye's optical resolution, sensitivity and field-of-view. Male and female fireflies may have different requirements in terms of the information they must extract from their visual environments. In rover fireflies, for example, males produce their species-specific flash while flying and searching for females . Females, who typically are not flying but rather are stationary on the ground or vegetation, detect a males' flashes and can respond with their own species-specific flash. The male, after seeing the female's flash response, must then orient to her so that he can land in her vicinity. The physical structure of firefly eyes provides a foundation for understanding the type of visual information that male and female fireflies must detect and/or process to carry out their visually-mediated behaviours. As facet area increases, for example, the light-gathering ability of the lens increases in a compound eye . However, resolution in terms of sampling frequency- not the optical cut-off frequency- decreases as the facet area increases.

The apparatus (Fig. 1) and methodology that we developed for this study provided us with the ability to efficiently measure and subsequently analyze the surface structures on the firefly compound eye. In spite of the small sample size used in this study, the results demonstrate that *P. carolinus* exhibits sexual dimorphism in the size of the eyes and specializations of regions of the eye.

In *P. carolinus*, there was a substantial size difference between the male and female eyes (Fig. 3). The male's eyes were larger by any measure, for example, surface area, number of facets, facet size, and angle subtended by the surface (Table 1). *P. carolinus* male eyes were two times the surface area of female *P. carolinus*. The differences between males and females may manifest themselves in differences in the visual fields of the eyes and the ability of the eye to resolve the location of visual stimuli. This suggests that the ability of males and females to detect the presence and position of visual stimuli are different. These data will be followed up with a more detailed analysis of a greater sample size.

Our systematic mapping of the eye also revealed regional consistent variation in facet size in males and females of *P. carolinus*. Significantly larger facets were found in the central region of the eyes. In other studies, such variations reflected specializations of the eye that were correlated with visual requirements to mediate important behaviours. The optical results predict properties of the eye important for behaviour and suggest behavioural and physiological experiments that can test these theories. We have not yet investigated the implications of larger facets in the central region of the firefly eye, but we suspect that this too will correlate to the firefly visually mediated behaviours.

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