

Peripheral eye dimensions in Longwing (*Heliconius*) butterflies vary with body size and sex but not light environment nor mimicry ring

BRETT M. SEYMOUR^{1,2}, W. OWEN McMILLAN², RONALD RUTOWSKI¹

¹ School of Life Sciences, Arizona State University, Tempe 85287, United States

² Tupper, Smithsonian Tropical Research Institute, Ciudad de Panama, Panama

brett.seymoure@asu.edu, mcmillano@si.edu, r.rutowski@asu.edu

Abstract. This study tests if tropical forest butterflies occupying similar light environments converge on eye morphology to meet shared demands of visual sensitivity. Total corneal surface area and facet diameters were measured and adjusted to body size for four species of *Heliconius* (Lepidoptera: Nymphalidae) butterflies that belong to two mimicry rings that frequent different light environments. Total corneal surface area and facet diameter differed among species, but not between mimicry rings and light environment. *Heliconius cydno* had the largest corneal surface areas, *H. erato* had the second largest, while *H. sapho* and *H. melpomene* did not differ from each other. *Heliconius cydno* and *H. erato* had larger facets than *H. cydno* and *H. melpomene*. Facet diameter was not linked to either mimicry ring or clade. Males had larger corneas relative to body size than females, but facet diameter did not differ by sex. As predicted, facet diameter differed by region of the eye. Lastly, we found that larger eyes had more facets. While the eyes of *Heliconius* generally seem to be larger than those of similarly sized butterflies, the hypothesis that light environment affects eye morphology was not supported and the finding that neither mimicry ring nor phylogeny explains facet diameter is perplexing, but suggests that adaptation to contrasting light environments might be instead found in the physiology of the visual system.

Keywords: Cornea, Eye size, Facet counts, Facet diameter, Mimicry.

INTRODUCTION

Many animals use vision to gather information about their surroundings (Lythgoe, 1979; Land & Nilsson, 2012). Their success in doing this depends on the match between their eye structure and the light available for visual processing. Irradiance, a measure of light available, is nine orders of magnitude greater on sunny days than on starlit nights (Johnsen, 2011). As expected, terrestrial species that live at the extremes of this continuum display very different eye structures with nocturnal animals showing features that enhance photon capture at the photoreceptors (Warrant, 2006; Frederiksen & Warrant, 2008;

Johnsen, 2011; Land & Nilsson, 2012). These features include larger eyes and facets than found in their diurnal relatives (Greiner *et al.*, 2004; Greiner, 2005; Warrant *et al.*, 2006; Somanathan *et al.*, 2008; Frederiksen & Warrant, 2008). Moreover, nocturnal and crepuscular species typically have superposition eyes in which a rhabdom (the microvilli component of the ommatidium's photoreceptors) is illuminated by light from several facet lenses enhancing sensitivity at the expense of resolution (Swihart, 1969; Horridge *et al.*, 1972; Warrant, 1999; Warrant *et al.*, 2004; Kelber, 2006). In contrast, diurnal insects (e.g. all non-skipper butterflies) often have apposition eyes in which the rhabdom in an ommatidium is illuminated only by light from the facet lens at the distal end of that ommatidium. Apposition eyes are much less sensitive than superposition eyes because photons from only one facet are caught by the individual photoreceptors.

Light environments that differ by several orders of magnitude in overall brightness can clearly lead to differences in eye morphology (i.e. night versus day), but how different are the eye features of diurnal animals that occupy habitats with smaller differences in available light (e.g. deep shaded forest vs. open field)? In this study, we test if eye morphology differs

Received: 7 July 2015

Accepted: 3 August 2015

Copyright: This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/3.0/> or send a letter to Creative Commons, 171 Second Street, Suite 300, San Francisco, California, 94105, USA.

among four related species of diurnal *Heliconius* (Klук) (Lepidoptera; Nymphalidae) butterflies that occur in light environments that can differ in brightness by one order of magnitude (Papageorgis, 1975; Endler, 1993; Estrada & Jiggins, 2002; B. Seymoure, unpublished data). This difference in brightness is relatively smaller than are differences in brightness encompassed by previous studies. For example, Frederiksen & Warrant (2008) compared the eyes of butterflies that fly at dusk to those that fly at midday when there is 100 times more light.

The four unpalatable species of *Heliconius* we studied include representatives of two different mimicry rings that occur in central Panama, the postman ring (*H. erato* and *H. melpomene*) and the blue-white ring (*H. cydno* and *H. sapho*; Brown, 1981; Chai, 1986). These two rings of Müllerian mimics occur in different microhabitats that present different light conditions (Gilbert, 1991; Mallet & Gilbert, 1995; Estrada & Jiggins, 2002; B. Seymoure, unpublished data). *Heliconius erato* and *H. melpomene* occur in more disturbed and open habitats, while *H. sapho* and *H. cydno* occur in established forest with full canopy cover (DeVries, 1987; Estrada & Jiggins, 2002; B. Seymoure, unpublished data). Endler (1993) quantified the differences in brightness (quantum flux) of forest understory and large open gaps in tropical forest in Panama. Large gaps, where *H. melpomene* and *H. erato* occur, are an order of magnitude brighter and are richer in long wavelengths than forest understory, where *H. cydno* and *H. sapho* occur (Endler, 1993; Estrada & Jiggins, 2002; B. Seymoure, unpublished data).

Do co-mimics share eye morphology that is adapted to shared environment and similar behaviors? Here, the results presented test the predictions that mimetic *Heliconius* butterflies that occur in darker environments (*H. sapho* and *H. cydno*) will have larger eyes and larger facets to improve sensitivity, while postman butterflies which live in more open environments will have smaller eyes and facets (Warrant, 2006). Note that the mimicry rings do not reflect phylogenetic relationships among these species (Brown, 1981; Kozak *et al.*, 2015; Figure 1). *Heliconius cydno* and *H. melpomene* are more closely related than *H. sapho* and *H. erato*. Hence, if recent common ancestry is an important determinant of eye morphology, it is predicted that eye morphology will be more similar within these pairs than among mimetic pairs.

Several patterns of variation in eye size and facet diameter in butterflies are known from previous studies (Ziemba & Rutowski, 2000; Rutowski, 2000; Merry *et al.*, 2006; Rutowski *et al.*, 2009). Eye size and facet diameter increase with body size, males typically have larger eyes than females, and facets in the frontal region of the eye tend to be larger than in other eye

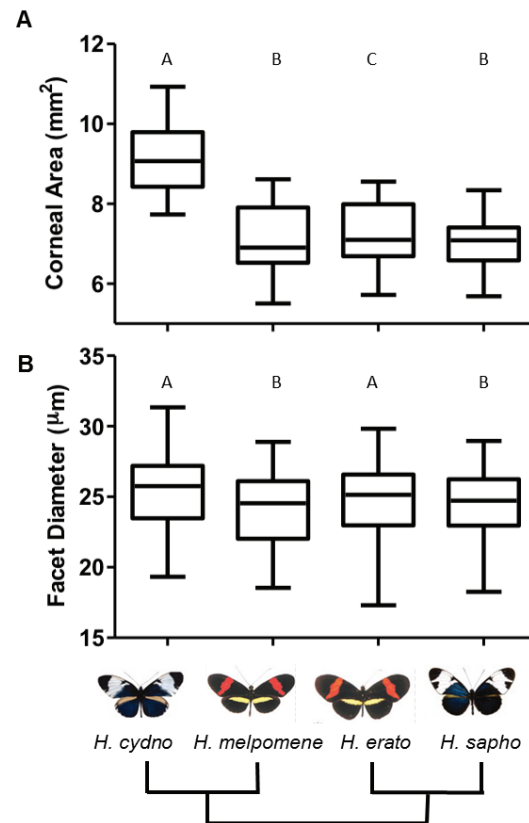


Figure 1. Interspecific differences in unadjusted eye morphology for the four *Heliconius* species studied. **A)** Absolute total corneal surface area. **B)** Mean absolute facet diameter. Letters (A, B, C) within each graph represent significantly different groups when controlling for body size. The data plotted here are not adjusted for body size unlike the statistical tests. Plots for each data set show the maximum and minimum values (upper and lower whisker, respectively), 1st and 3rd quartiles (top and bottom of box, respectively), and the mean (horizontal line within box). Phylogenetic relationships among these species are shown at the bottom (Brown 1981; Kozak *et al.* 2015). Note that *H. melpomene* and *H. erato* are found in brighter environments than *H. cydno* and *H. sapho*.

regions (Land, 1997; Rutowski, 2009). Hence, our analysis took into consideration both size and sex of all sampled individuals and included measurements from several eye regions.

MATERIAL AND METHODS

Specimen collection

Ninety-two adult *Heliconius* butterflies were collected for measurements in Parque de Nacional Soberanía in Panama from February to May 2013

Table 1. Sample sizes, body area measurements, and total corneal surface area for the *Heliconius* species studied. Means are given with standard deviations

Species	Sex	N	Forewing (mm)	Femur (mm)	PCI	Cornea (mm ²)
<i>H. cydno</i>	M	12	39.6±1.76	4.57±0.34	-1.17±0.87	9.45±0.90
	F	10	39.7±2.21	4.46±0.41	-0.41±1.84	8.77±0.78
<i>H. melpomene</i>	M	12	34.8±3.19	4.26±0.37	0.32±1.07	7.32±1.03
	F	12	35.2±1.44	4.04±0.31	0.67±0.95	6.79±0.56
<i>H. sapho</i>	M	11	36.8±3.32	4.06±0.44	-0.77±0.77	7.31±0.66
	F	12	38.8±1.51	4.34±0.27	0.67±.66	6.76±0.43
<i>H. erato</i>	M	12	32.5±2.26	3.61±0.30	1.37±1.09	7.27±0.93
	F	11	34.7±2.28	3.74±0.37	0.68±1.19	7.21±0.77

(Table 1). Adults with little wing wear were netted and then stored in glassine envelopes for transportation to lab facilities in Gamboa, Panama, where the butterflies were euthanized by freezing.

Body size covariate

As measures of body size we used hind femur length and forewing length of each individual measured with digital calipers to the nearest 0.01 mm (Rutowski, 2000; Rutowski *et al.*, 2009). Principal component analysis on these two measures revealed a first principal component that explained 90% of variation (hind femur length factor loading = -0.707; forewing length loading = -0.707). This component was used as a covariate representing body size in our analyses.

Cornea preparation

The head of each individual was severed from the thorax and the antennae, proboscis, and labial palps were removed. Following the methods of Ziemba and Rutowski (2000), the heads were soaked in 20% NaOH for 18 to 24 h to loosen the tissues behind the cuticular cornea. Once the soft tissues were removed, the cornea was cut along the dorsal-ventral axis and then laid flat on a microscope slide. A coverslip was placed over the cornea and then preserved and sealed with Cytoseal 60 (Richard-Allan Scientific, Kalamazoo, MI). These prepared slides were air dried for 24 h before being photographed.

Total corneal surface area measurements

Corneal squashes were photographed at approximately 20x magnification with a microscope (model MZM1, Askania Mikroskop Technik Rathenow, Germany) fitted with an OptixCam (Summit Series, The Microscope Store, Roanoke, VA) run with OCView Software (The Microscope Store, Roanoke, VA). A photograph taken of a micrometer scale was used to calibrate measurements made from other images. Total corneal surface area was measured by one observer in ImageJ with the lasso tool (Rasband, 2012); repeatability of these measurements was very high (intraclass correlation coefficient = 0.998).

Facet diameter measurements

Diameter of facets was measured in each of six regions of the eye: posterior, dorsal, anterior, anteroventral, ventral, and lateral (Figure 2). For these measurements, mounted corneas were photographed with the OptixCam attached to a compound microscope (Spencer Phase Star, American Optical, Hicksville, NY) at 100x magnification. The photographs were calibrated with a slide micrometer and all measurements were made within ImageJ. Within each region of each eye, distance was measured across ten facets in a row in two separate locations at least ten facets apart. The distance for each location was divided by ten to get an average facet diameter for each location. Then the two locations in each region were averaged to provide an average facet diameter for each region. As with total corneal

surface area measurements, one observer measured facet diameters and again repeatability was very high (intraclass correlation coefficient = 0.984).

Facet counts

To further understand the eye morphology of *Heliconius* butterflies, the number of facets were counted for two individuals for each sex and species. Utilizing the total corneal surface area photographs, the cell counter plugin in ImageJ was used for counting the number of facets. We selected photos where all facets were easily countable.

Statistical analyses

Body size principal components were calculated in R (R Development Core Team, 2008). All other tests were run in SPSS version 19 (IBM, Armonk, NY). Total corneal surface area was analyzed using a three-way nested analysis of covariance (ANCOVA). The covariate was PC1 of body size, the between factors were sex, mimicry ring, clade membership, and species. Species was nested both within mimicry ring and clade membership. Facet diameter was analyzed using repeated-measures ANCOVA. The facet diameters for each region of the eye were the within factor, and PC1 of body size served as the covariate. Sex, mimicry ring, and clade membership were the between factors, and again, species was nested within mimicry ring and clade membership. For both tests, post-hoc Helmert contrasts were implemented to determine differences among groups. All statistical inferences were made at the 0.05 level of significance.

RESULTS

Total corneal surface area

As in other species of butterflies, total corneal surface area scaled positively with body size (ANCOVA, $F_{1,97}=48.515$, $p<0.001$; Figure 3) and males had larger eyes than females independent of body size ($F_{1,97}=20.42$, $p<0.001$; Figure 4). However, further Helmert analysis revealed that there was a significant difference between the sexes for total corneal surface area for *H. sapho* ($p=0.004$), and *H. cydno* ($p=0.038$), but corneal surface area did not differ by sex for *H. melpomene* ($p=0.067$) and for *H. erato* ($p=0.332$). Within each sex of each species there was a strong negative allometry in the relationship between eye size and body size, which means small individuals had relatively larger eyes compared to their larger counterparts (Figure 3).

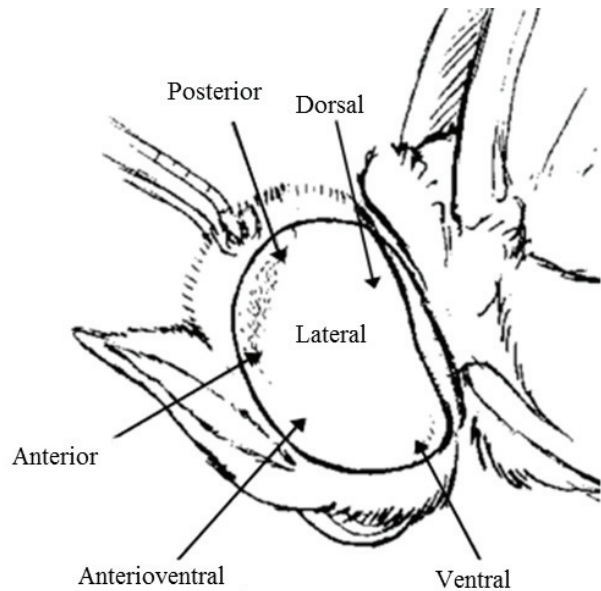


Figure 2. Eye regions in which facet diameter was measured. Figure modified from Rutowski (2000) and Merry *et al.* (2006).

Body-size-adjusted corneal surface area of *H. cydno* and *H. erato* were significantly different from each other and the other two species ($F_{3,47}=46.365$, $p<0.001$). Specifically, Helmert contrasts revealed that *H. cydno* had the largest eyes ($p<0.001$) while *H. erato* had the second largest ($p<0.001$; Figure 1). *H. sapho* and *H. melpomene* did not differ from one another and had the smallest eyes ($p=0.064$; Figure 1). Contrary to our prediction, there was no difference in total corneal surface area between the two mimicry rings ($F_{1,97}=0.510$, $p=0.477$) but the effect of clade was significant ($F_{1,97}=40.394$, $p<0.001$).

Facet diameter

As expected from studies of other butterflies, facet diameters differed among eye regions (ANCOVA with Greenhouse-Geisser correction, $F_{3,95}=210.39$, $p<0.001$; Figure 5). Lateral facets were the largest, anterior and anterioventral facets were next largest in diameter; then facets became smaller from posterior to ventral to dorsal. Body size positively predicted facet diameter (ANCOVA, $F_{1,97}=11.295$, $p=0.001$; Figure 6), but facet size did not differ by sex ($F_{1,97}=0.829$, $p=0.365$), mimicry ring ($F_{1,97}=0.001$, $p=0.970$), or phylogeny ($F_{1,97}=0.775$, $p=0.381$). Facet size differed among species ($F_{3,47}=7.438$, $p=0.001$; Figure 1B). As with total corneal surface size, *H. sapho* and *H. melpomene* had similarly smaller facets ($p=0.472$) than *H. cydno* and

Table 2. Facet diameter by region of the eye as a function of species and sex. Means are given with standard deviations.

Species	Sex	N	Facet Diameter (μm)					
			Posterior	Ventral	Dorsal	Anterior	Lateral	Anterioventral
<i>H. cydno</i>	M	12	24.9 \pm 1.43	23.9 \pm 0.90	21.2 \pm 1.09	26.4 \pm 1.14	27.2 \pm 0.98	27.0 \pm 1.44
	F	10	24.6 \pm 1.48	25.3 \pm 1.51	21.1 \pm 1.18	26.9 \pm 0.78	27.5 \pm 2.05	27.7 \pm 0.64
<i>H. melpomene</i>	M	12	23.8 \pm 1.17	23.5 \pm 1.80	21.0 \pm 1.03	26.2 \pm 1.96	26.0 \pm 2.03	26.5 \pm 1.53
	F	12	23.9 \pm 1.29	22.3 \pm 1.50	20.9 \pm 1.75	24.9 \pm 1.79	25.9 \pm 1.23	24.3 \pm 2.34
<i>H. sapho</i>	M	11	24.6 \pm 1.04	23.9 \pm 1.25	20.8 \pm 1.69	25.8 \pm 0.93	26.2 \pm 1.23	25.5 \pm 1.93
	F	12	24.3 \pm 2.02	23.3 \pm 1.43	21.4 \pm 1.50	26.1 \pm 1.13	26.2 \pm 1.52	26.0 \pm 1.81
<i>H. erato</i>	M	12	23.4 \pm 1.50	22.8 \pm 2.15	21.5 \pm 1.79	26.7 \pm 1.18	27.1 \pm 1.42	26.3 \pm 1.98
	F	11	24.3 \pm 1.93	23.6 \pm 1.84	21.7 \pm 2.29	26.2 \pm 1.09	26.7 \pm 1.47	25.1 \pm 1.48

H. erato, which had the largest facet diameters and did not differ from one another ($p=0.639$). The data were suggestive of a three-way interaction of region by sex by species (ANCOVA, $F_{15,243}=1.71$, $p=0.051$). And as with total corneal surface area and body size, there was a strong negative allometry in the relationship between facet diameter and body size (Figure 6).

Facet counts

Facet number was highly positively correlated with total corneal surface area ($R^2=0.92$ for males and $R^2=0.73$ for females; Figure 7). The largest corneas had the most facets and the smallest corneas had the fewest facets (Table 2). Males have absolutely larger eyes than females and therefore have more facets.

DISCUSSION

Eye size varies with body size

Previous research has shown that eye size in Lepidoptera increases with body size (Yagi & Koyama, 1963; Rutowski, 2000; Rutowski *et al.*, 2009) and the *Heliconius* species examined here are no different. Here we found that larger *Heliconius* individuals have larger total corneal surface area and larger facets. However, we found the rate with which eye size changes with body size is much lower in *Heliconius* than reported for other butterflies (Rutowski, 2000; Figures 3 & 6). The very negatively allometric relationships between body size and eye

size are unexpected and suggest selective pressures on *Heliconius* that favor development of large eyes regardless of body size. Regardless of the degree of allometry, eye performance is related to body size and depends on eye shape, facet number and facet size (Land, 1989; Land, 1997; Zollikofer *et al.*, 1995). Therefore, larger *Heliconius* butterflies should have increased sensitivity, acuity, larger visual fields or a combination of these characteristics (Rutowski, 2000; Frederikson & Warrant, 2008).

Interestingly, all of the *Heliconius* species we examined have a higher corneal surface area to body size ratio than that reported for other butterflies (Rutowski, 2000; Rutowski *et al.*, 2009). Rutowski (2000) found that the corneal surface area to body size ratio is close to 1:1 for 16 different species of butterflies with lower ratios of 1:2 and higher ratios of 11:10. Here we found corneal surface area to body size ratios greater than 2:1, indicating that *Heliconius* have the largest eyes relative to body size of butterflies studied thus far.

Larger total corneal surface areas could have several effects on vision including a larger visual field (ommatidia pointing in a larger number of directions), more acute and sensitive vision, or both. Visual field dimensions of butterflies are generally huge and do not change much with body size (Rutowski *et al.*, 2009). There is no reason to think this will not also be true for *Heliconius*. However, in *Heliconius* the number and diameter of facets do increase with body size. So, given no change in visual field dimensions, the increase in cornea size and in facet number should mean overall

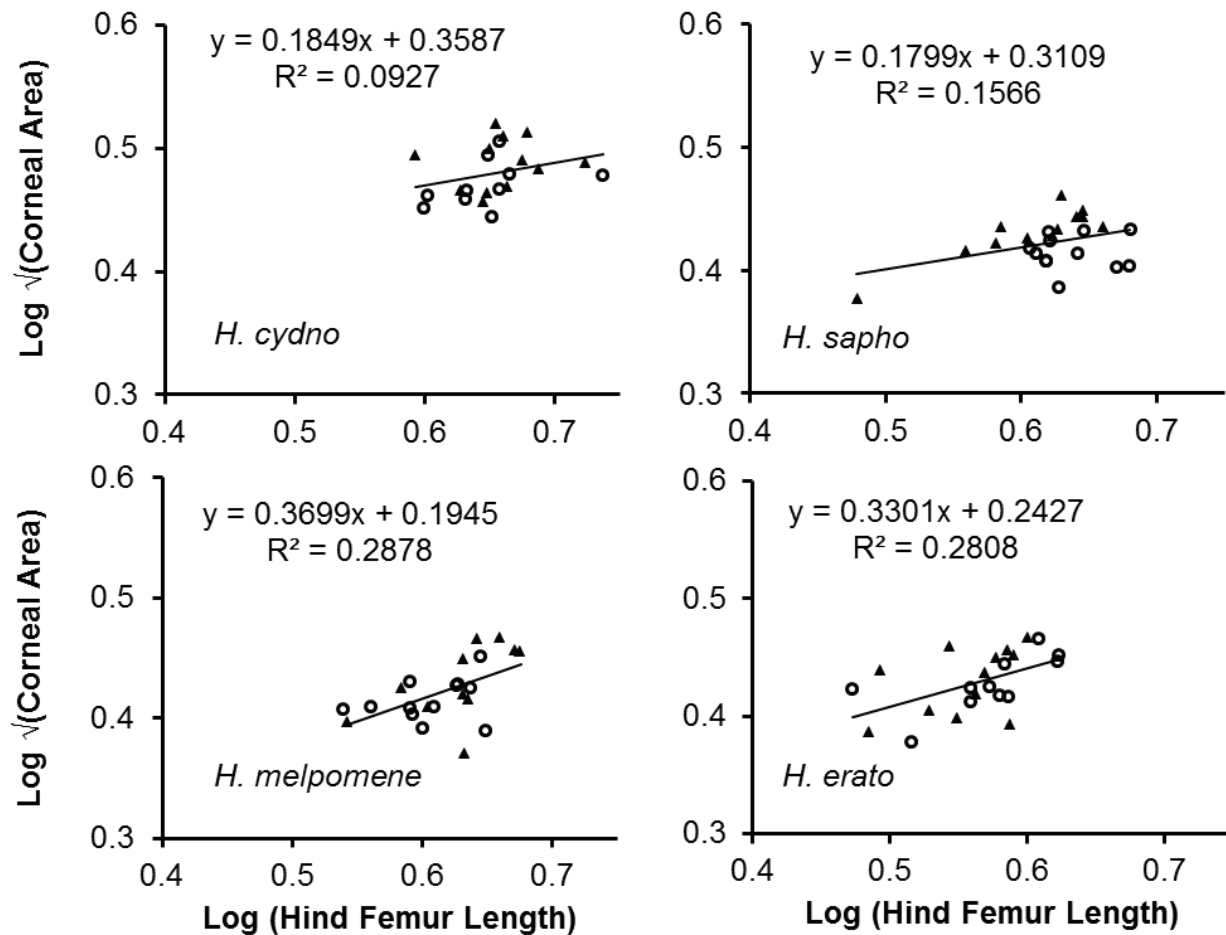


Figure 3. The relationship between eye size and body size as measured by hind femur length for each sex of each species (triangles, males; open circles, females). The y-axis represents the log of the square root of the total corneal surface area and the x-axis represents the log of hind femur length. The double-logarithmic plot is used to determine if the relationship between total corneal surface area and hind femur length is allometric. A slope of 1 would indicate an isometric relationship between body size (hind femur length) and eye size (total corneal surface area). However, the slopes here indicate that eye size has a very negative allometric relationship with body size.

lower inter-ommatidial angles in larger eyes. Similarly, the increase in facet diameter with body size will mean a higher photon catch per ommatidium such that larger eyes should be more sensitive. Hence, *Heliconius* should have better low light vision than most other butterflies in the same body size range. What selective pressures might have driven this divergence is not clear. Perhaps it is that they frequent forest shade (i.e. low light) environments which makes visual detection and recognition tasks more demanding than those of butterflies in environments with higher light levels. Interestingly in Rutowski *et al.* (2009) the species examined in the *Heliconius* size range, *P. sylvia*, with its relatively smaller facets frequents open environments with high light levels.

Blue-white males have larger eyes than females

Previous studies showed that male Lepidoptera have larger corneas and facets than conspecific females (Yagi & Koyama, 1963; Ziemba & Rutowski, 2000; Rutowski, 2000; Lund *et al.*, 2001). Blue-white males had larger eyes than females when controlled for body size, but postman individuals did not differ in eye size between species. Why only blue-white individuals would have an intraspecific difference is intriguing because other studies hypothesize that male Lepidoptera have generally larger eyes as a result of the visual demands of finding mates (Yagi & Koyama, 1963; Rutowski, 2000).

Facet diameter varies by region

The largest facets in butterflies are in the anterior regions of the eyes for maintaining flight and for locating and recognizing food resources, mates, and larval host plants (Land, 1997; Rutowski & Warrant, 2002; Rutowski, 2003; Rutowski *et al.*, 2009). We observed a similar pattern in the *Heliconius* species studied here but with large facets also in the anterioventral and lateral eye regions. Unlike in previous studies (Rutowski & Warrant, 2002; Rutowski *et al.*, 2009), there were no differences in facet diameters among the sexes or mimicry rings. This again supports the notion that vision may function similarly in males and females of *Heliconius* butterflies.

The lateral facets, located in the center of the cornea, are the largest for all four species, which contrasts with previous reports that largest facets in butterflies are found anteriorly and anterioventrally, most likely for locating and recognizing both host plants and mates (Merry *et al.*, 2006; Rutowski *et al.*, 2009). Large lateral facets may enhance processing of optic flow in flight, the pattern of apparent motion of elements in the visual scene as the observer moves (Srinivasan *et al.*, 2000). The greatest angular velocity of objects in the visual scene of a flying butterfly will be in the lateral regions and thus the lateral optical flow is most likely to suffer from visual blur which will be minimized when photon flux and signal to noise ratios are high. These conditions will happen when facets are large, such as they are in the lateral regions of the eye. Of course, this explanation warrants testing and further comparative research on compound eyes and optic flow is needed.

Larger eyes have more but not larger facets

Very little is known about the relationship between eye size and facet number for the Lepidoptera. Ziemba & Rutowski (2000) found that although eye size differs between males and females in the butterfly *Asterocampa leilia*, the number of facets per eye was the same in males and females. Males of *A. leilia* have larger facets than females, which leads to a larger eye size without more facets. Unlike *A. leilia*, in *Heliconius* the sexes differ in the number of facets per eye. Furthermore, eye size correlates with facet number with similar negative allometry to body size as was found with corneal surface area and body size. Again, this negative allometry is likely due to selection for very large facets regardless of body size and because larger eyes have more facets instead of larger facets, a very negative allometric relationship

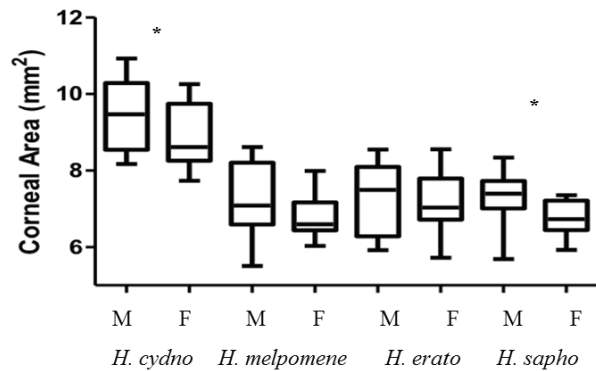


Figure 4. Absolute total corneal surface area for each sex of each species. See legend in Figure 1 for further details of the box-and-whisker plots. The asterisks mark intraspecific sexual differences that were significant at the 0.05 level.

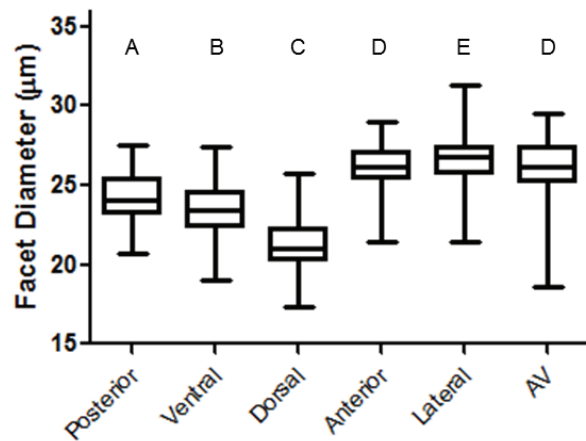


Figure 5. Mean facet diameter across different regions of the eye for all individuals of all species (n=92). Letters represent significantly different groups when body size is a covariate. Only anterior and anterioventral regions are not statistically different from one another.

would be predicted. This finding is comparable to what has been found in eusocial hymenoptera in which the larger the eye, the greater number of facets (Jander & Jander, 2002; Streinzer *et al.*, 2013).

Eye morphology, mimicry ring and light environment

The predictions about the relationship between mimicry rings, which correspond to light environment, and eye features were not supported. One possible reason for this result is that differences in light

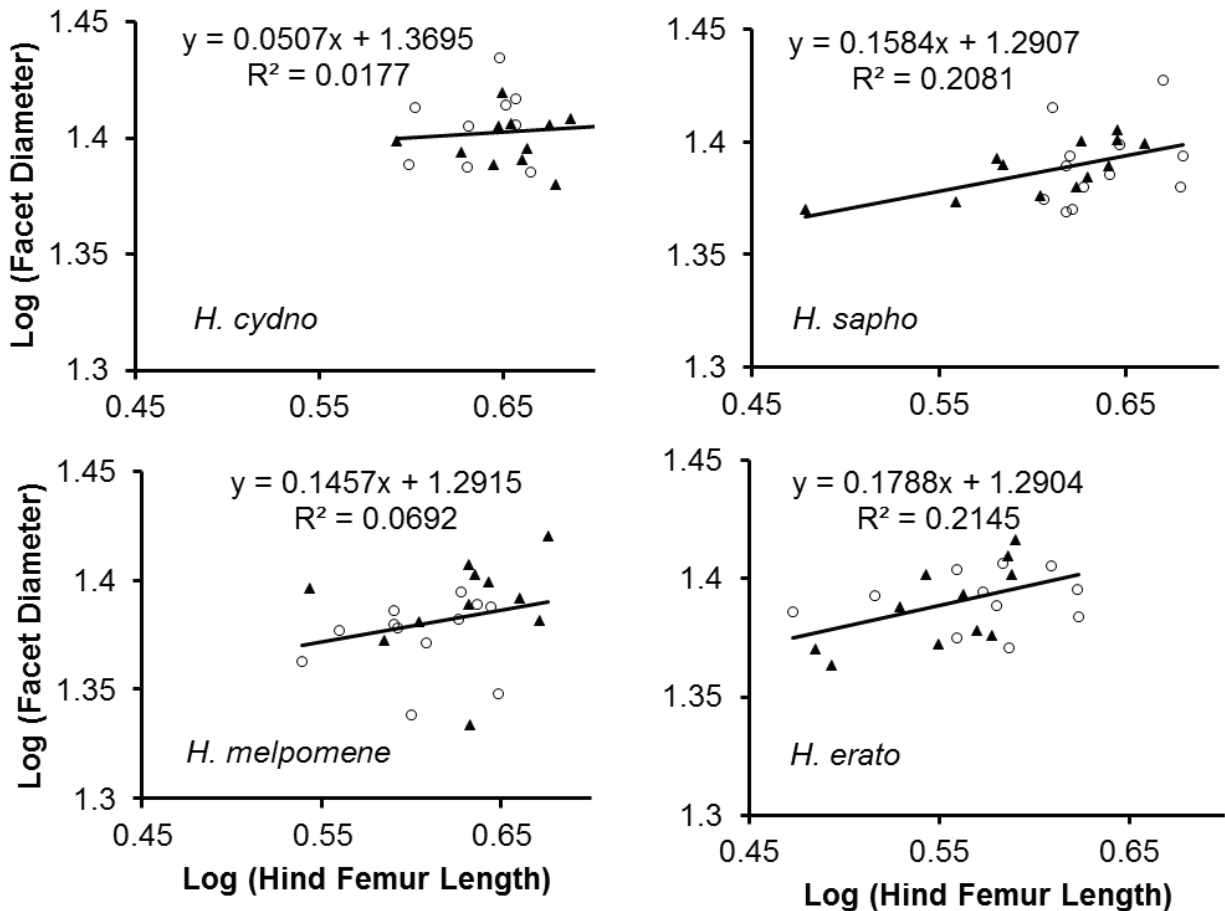


Figure 6. The relationship between facet diameter and body size as measured by hind femur length for each sex of each species (triangles, males; open circles, females). See figure 3 for explanation of the double-logarithmic plots.

intensity where these species typically occur are too small to have shaped the peripheral features of eye morphology that we examined. Preliminary results from electroretinograms of these butterflies reveal that the blue-white butterflies that live in forest shade environments have greater absolute sensitivity (i.e. can see in darker environments) than the postman butterflies which live in very open environments (B. Seymoure *et al.*, unpublished). Because these two groups did not differ in the measures of eye structure reported here, physiological differences in eye performance between animals that live in different light environments are expected to be the result of differences in eye structure other than those measured here.

Apposition compound eyes can be rendered more sensitive through a pupil mechanism, by lengthening and/or widening the rhabdoms or through spatial and/or temporal summation of responses to dim light signals (Jonson *et al.*, 1998; Warrant *et al.*, 2004;

Greiner *et al.*, 2005; Warrant, 2006; Land & Nilsson, 2012). In fact, Jonson *et al.* (1998) revealed that butterflies that occur in different light environments vary in pupil response with dim habitat species having a pupil mechanism that restricts photons entering the rhabdom in much dimmer environments than bright habitat species. Furthermore, Frederiksen & Warrant (2008) found that the crepuscular Owl butterfly (*Caligo memnon*) has four times the sensitivity of a similar sized diurnal butterfly that stems from not only increased facet diameters, but also wider rhabdoms and neural summation. Perhaps *Heliconius* individuals in darker environments have similar features that increase sensitivity. This is currently under investigation in our lab (B. Seymoure *et al.*, unpublished).

This work reveals several potentially fruitful research directions into the visual ecology and behavior of *Heliconius* butterflies. This study only investigated the eye morphology of four of the 44

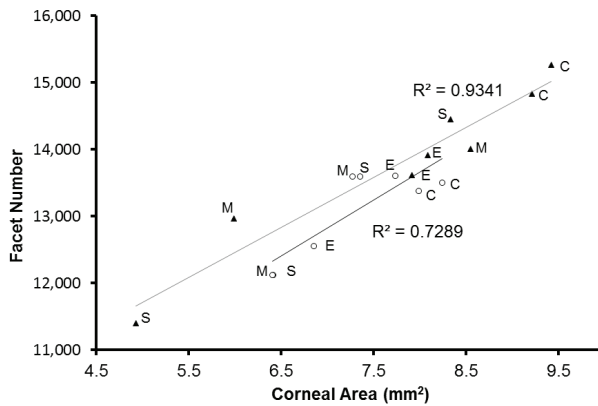


Figure 7. Relationship between facet number and total corneal surface area (mm^2) for selected *Heliconius* males and females. Letters near data points represent species: S = *H. sapho*, M = *H. melpomene*, E = *H. erato*, C = *H. cydno*. Lines represent least squares regression for males (closed triangles) and females (open circles).

Heliconius species and further *Heliconius* research is needed to understand why these species differ drastically from other butterflies and the role of ancestry in eye morphology. Furthermore, to understand how light environment has affected compound eye morphology, compelling studies could include phylogenetically-controlled comparisons of eye structure of diurnal species that differ in the light environments where they tend to occur. Such studies might also include a larger array of eye features including inter-ommatidial angles, visual field dimensions, pupillary responses, rhabdom lengths as well as physiological recordings such as electroretinograms or intracellular recordings. Such studies are currently underway in our lab and will shed light on the nature and tuning of visual adaptations in insects that occur in diverse light environments.

ACKNOWLEDGEMENTS

This work was supported by Arizona State University and the Smithsonian Tropical Research Institute (STRI). B. Seymoure was funded by a STRI Short Term Fellowship, Grants in Aid of Research from Sigma Xi and the Society for Integrative and Comparative Biology, and the Arizona State University and Smithsonian Tropical Research Institute Partnership. A travel grant from the Lepidoptera Research Foundation enabled B. Seymoure to present preliminary findings of this work and receive valuable feedback. Andrew Raymundo and Ben Rice provided crucial assistance in the field. Rachel Olzer, Kaci Fankhauser, Britney Southers and Jennifer Armstrong were helpful with data collection. We appreciate logistical support from R. Urriola, S. Van Bael, A. Tapia, A. Bilgray, B. van Schooten and comments on the project and manuscript from K. McGraw, B. Jones, R. Ligon, K. Pegram,

N. Lessios, and from the Rutowski and McGraw labs at ASU. For the collecting permit (SE/A-7-13), we thank Autoridad Nacional del Ambiente (ANAM) of the Republic of Panama.

DISCLOSURE

The authors have no conflicts of interest, including specific financial interests, relationships and affiliations relevant to the subject of this manuscript.

LITERATURE CITED

- BENSON, W.W., K.S. BROWN & L.E. GILBERT. 1975. Coevolution of plants and herbivores: Passion flower butterflies. *Evolution* 29: 659-680.
- BROWN, K.S.J. 1981. The biology of *Heliconius* and related genera. *Annual Review of Entomology* 26: 427-456.
- CHAI, P. 1986. Field observations and feeding experiments on the responses of rufous-tailed jacamars (*Galbula ruficauda*) to free-flying butterflies in a tropical rainforest. *Biological Journal of the Linnean Society* 29: 161-189.
- DEVRIES, P.J. 1987. *Butterflies of Costa Rica and their natural history*. Princeton University Press, New Jersey.
- ENDLER, J.A. 1993. The color of light in forests and its implications. *Ecological Monographs* 63: 1-27.
- ESTRADA, C. & C. JIGGINS. 2002. Patterns of pollen feeding and habitat preference among *Heliconius* species. *Ecological Entomology* 27: 448-456.
- FREDERIKSEN, R. & E.J. WARRANT. 2008. Visual sensitivity in the crepuscular owl butterfly, *Caligo memnon* and the diurnal blue morpho, *Morpho peleides*: a clue to explain the evolution of nocturnal apposition eyes? *The Journal of Experimental Biology* 211: 844-851.
- GILBERT, L.E. 1991. Biodiversity of Central American *Heliconius* community: Pattern, process and problems. *In*: Price, P.W., Lewinsohn, T.M., Fernandes, G.W., and Benson, W.W. (Eds), *Plant-animal interaction: Evolutionary ecology in tropical and temperate regions*. John Wiley and Sons, London, pp. 403-427.
- GREINER, B. 2005. Visual adaptations in the night active wasp *Apoica pallens*. *Journal of Comparative Neurology* 495: 255-262.
- GREINER, B., W.A. RIBI, & E.J. WARRANT. 2004. Retinal and optical adaptations for nocturnal vision in the halictid bee *Megalopta genalis*. *Cell Tissue Research* 316: 377-390.
- GREINER, B., W.A. RIBI & E.J. WARRANT. 2005. A neural network to improve dimlight vision? Dendritic fields of the first-order interneurons in the nocturnal bee *Megalopta genalis*. *Cell Tissue Research* 322: 313-320.
- HORRIDGE, G.A., C. GIDDINGS, & G. STANGE. 1972. The superposition eye of skipper butterflies. *Proceedings of the Royal Society London Biological Society* 182: 457-495.
- JANDER, U. & R. JANDER. 2002. Allometry and resolution of bee eyes (Apoidea). *Arthropod Structure and Development* 30: 179-193.
- JOHNSON, S. 2011. *Optics of life*. Princeton University Press, Princeton.
- JONSON, A.C.J., M.F. LAND, D.C. OSORIO, & D.-E. NILSSON. 1998. Relationships between pupil working range and habitat luminance in flies and butterflies. *Journal of Comparative Physiology A* 182: 1-9.
- KELBER, A. 2006. *Invertebrate colour vision*. Invertebrate Vision. Cambridge University Press.
- KOZAK, K.M., N. WAHLBERG, A.F.E. NEILD, K.K. DASMAHAPATRA, J. MALLET & C. JIGGINS. 2015. Multilocus species trees show the recent adaptive radiation of the mimetic *Heliconius* species. *Systematic Biology* 64: 505-524.

- LAND, M.F. 1989. Variations in the structure and design of compound eyes. *Facets of Vision*. Berlin; Springer.
- LAND, M.F. 1997. Visual acuity in insects. *Annual Reviews of Entomology* 42: 147-177.
- LAND, M.F. & D.E. NILSSON. 2012. *Animal eyes*. Oxford University Press.
- LUND, N., E. CWENGROS, & R.L. RUTOWSKI. 2001. Sexual dimorphism in eye morphology in *Eucheira socialis* (Pieridae). *Journal of the Lepidopterists' Society* 55: 74-77.
- LYTHGOE, J.N. 1979. *The ecology of vision*. Clarendon Press, Oxford.
- MALLET, J. & L.E. GILBERT. 1995. Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. *Biological Journal of the Linnean Society* 55: 159-180.
- MERRY, J.W., N.I. MOREHOUSE, K. YTURRALDE, & R.L. RUTOWSKI. 2006. The eyes of a patrolling butterfly: Visual field and eye structure in the Orange Sulphur, *Colias eurytheme* (Lepidoptera, Pieridae). *Journal of Insect Physiology* 52: 240-248.
- PAPAGEORGIS, C. 1975. Mimicry in Neotropical butterflies. *American Scientist* 63: 522-532.
- R DEVELOPMENT CORE TEAM. 2008. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- RASBAND, W.S. 1997-2012. *ImageJ*. U. S. National Institutes of Health, Bethesda, Maryland, USA. Available at <http://imagej.nih.gov/ij/>.
- RUTOWSKI, R.L. 2000. Variation of eye size in butterflies: inter- and intraspecific patterns. *Journal of Zoology* 252: 187-195.
- RUTOWSKI, R.L. 2003. Visual ecology of adult butterflies. *In: Boggs, C., Watt, W., Ehrlich, P.R. (Eds.), Butterflies: Ecology and evolution taking flight*. University of Chicago Press, Chicago, pp. 9-25.
- RUTOWSKI, R. L. & E. WARRANT. 2002. Visual field structure in the Empress Leilia, *Asterocampa leilia*: dimensions and regional variation in acuity. *Journal of Comparative Physiology A* 188: 1-12.
- RUTOWSKI, R.L., L. GISLÉN, & E.J. WARRANT. 2009. Visual acuity and sensitivity increase allometrically with body size in butterflies. *Arthropod Structure & Development* 38: 91-100.
- SOMANATHAN, H., R. BORGES, E. WARRANT, & A. KELBER. 2008. Visual ecology of Indian carpenter bees I: Light intensities and flight cavity. *Journal of Comparative Physiology A* 194: 97-107.
- SRINIVASAN, M.V., S.W. ZHANG, M. ALTWEIN & J. TAUTZ. 2000. Honeybee navigation: nature and calibration of the "odometer." *Science* 287: 851-853.
- STREINZER, M., A. BROCKMANN, N. NAGARAJA, & J. SPAETHE. 2013. Sex and caste-specific variation in compound eye morphology of five honeybee species. *PLoS ONE* 8:e57702.
- SWIHART, S.L. 1969. Colour vision and the physiology of the superposition eye of a butterfly (Hesperiidae). *Journal of Insect Physiology* 15: 1347-1365.
- WARRANT, E.J. 1999. Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. *Vision Research* 39: 1611-30.
- WARRANT, E.J. 2006. *Invertebrate vision in dim light*. Invertebrate Vision. Cambridge University Press.
- WARRANT, E.J., KELBER, A., GISLÉN, A., GREINER, B., RIBI, W. AND WCISLO, W.T. 2004. Nocturnal vision and landmark orientation in a tropical halictid bee. *Current Biology* 14: 1309-1318.
- YAGI, N. & N. KOYAMA. 1963. *The compound eye of Lepidoptera: Approach from organic evolution*. Shinkyō Press, Tokyo.
- ZIEMBA, K.S. & R.L. RUTOWSKI. 2000. Sexual dimorphism in eye morphology in a butterfly (*Asterocampa leilia*). *Psyche* 103: 25-36.
- ZOLLIKOFFER, C., R. WEHNER & T. FUKUSHI. 1995. Optical scaling in conspecific Cataglyphis ants. *Journal of Experimental Biology* 198: 1637-1646