

SEXUAL DIMORPHISM IN EYE MORPHOLOGY IN A BUTTERFLY (*ASTEROCAMPA LEILIA*; LEPIDOPTERA, NYMPHALIDAE)

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ABSTRACT

In the Empress Leilia butterfly, *Asterocampa leilia*, as in many insects, males have larger eyes than females. We explore the morphological causes and consequences of this dimorphism in eye size by comparing the corneal surface area, facet numbers, and patterns of variation in facet dimensions in males and females. We report that, with body size (measured by forewing length) controlled, male eyes are consistently larger than female eyes, and that, although males and females do not differ significantly in the number of facets per eye, males have significantly larger facets. Also, males have disproportionately larger facets both frontally and dorsally. As a result of these sexual differences in eye structure, males are expected to have a larger and more acute visual field than females which could be advantageous in the context of this species' mate searching tactic.

INTRODUCTION

In many insect species, males have larger compound eyes with a larger corneal surface area than females (Land 1989, 1990). These sexual differences in eye surface area are due to differences in facet number, facet size or a combination of both. A facet is the lens that gathers light for the underlying ommatidium or photoreceptive element. There may be hundreds or thousands of ommatidia and, therefore, facets in a compound eye, which together make up the cornea of the eye.

Males are expected to have a higher optical resolution and sensitivity, or a larger visual field than females depending on exactly how facet characteristics differ. First, if male eyes are larger because the facets are larger, especially in certain eye regions, the resolution and light sensitivity of the eye will be greater in those regions, which are

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called acute zones (Land 1990; Warrant and McIntyre 1992). In acute zones, the angles between the optical axes of adjacent ommatidia are usually smaller than in the rest of the eye, which also contributes to the enhanced resolving power of these regions (Land 1989). The location of acute zones is often correlated with the insect's lifestyle and behavior (Wehner 1981). For example, male insects often have acute zones in eye regions that are used in locating and tracking mates (Land 1989).

Second, if larger eyes have only more and not larger facets, and the interommatidial angles stay the same, size of the visual field will be enlarged but resolution will not change. Under these conditions additional corneal surface may increase binocular overlap or reduce blind spots (the regions of space that are not sampled by any ommatidia).

In this study we have examined the morphological concomitants of sexual differences in eye size in a butterfly, *Asterocampa leilia*. Preliminary observations suggested that *A. leilia* males have larger eyes than females, but it is not known what structural differences account for this dimorphism. We are also interested in why males have evolved larger eyes than females. Given that eye size may be related to visual system performance, sexual dimorphism in eye size is expected to reflect sexual differences in behavior or lifestyle that make different demands on eye performance. Males of *A. leilia* employ a sit-and-wait tactic to locate visually potential mates. Their perch sites are on or near the larval food plant, desert hackberry (*Celtis pallida*) (Rutowski and Gilchrist 1988; Rutowski et al. 1991). Early in the day, males perch on the ground in open areas near hackberry trees. Thermoregulatory concerns later in the day, however, cause males to seek cooler perches in shade or about one meter up on the trees (Rutowski et al. 1994).

If the size dimorphism is due to selection acting on males in the context of mate location, we expect that the enlarged eyes of *A. leilia* males are due to enlarged facets directed dorsally and frontally because males look either up from the ground or out of a bush to locate potential mates. Forward directed acute zones would be advantageous to males perched on the plant looking out at passing females, while dorsal acute zones would be advantageous for males perched on the ground looking up at passing females.

With this evolutionary explanation in mind we have studied the structural details of eye morphology in males and females of *A. leilia*. First, we quantified eye size in males and females using direct measurements of the corneal surface area. Then, we counted and measured facets to determine which factor or factors contribute to the sexual

dimorphism in eye size. Lastly, we examined how facet size varies across the eye surface to see if there is evidence of acute zones.

MATERIALS AND METHODS

Forty specimens of *A. leilia* (19 females and 21 males) were obtained from a field site in the Sonoran Desert near Sycamore Creek, approximately 50 miles northeast of Phoenix, Arizona. We preserved each butterfly by freezing and measured its forewing length, an indicator of body size, with digital clippers to the nearest 0.1 mm from the anterior point of attachment to the wing's apex.

The head was removed from each specimen and soaked in 10% NaOH for 30 minutes to soften the tissue behind the cornea. The cornea was then pulled away from the underlying tissues in each individual's left eye, and a series of radial cuts was made in from the corneal perimeter so that we could flatten the cornea on a microscope slide. The cuts were positioned so that they indicated the dorso-ventral and anterior-posterior axes. Each cornea was mounted in glycerol on a microscope slide and covered with a coverslip whose edges were sealed with Cytoseal 60 mounting medium.

Facet counts: Each cornea was photographed using a Nikon Optiphot compound microscope equipped with a 35mm camera. Facets were counted from xerographic enlargements of the prints.

Surface area measurements: Eye surface area measurements were made using Bioscan Optimas Image analysis software that received images from a Sanyo CCD video camera. Each image was enhanced to make the eye border clear. This permitted automatic tracing of the eye border and calculation of corneal surface area. The system was pre-calibrated by imaging a stage micrometer.

Facet area measurements: Facet areas were also measured by computer, a Macintosh Centris 650 system equipped with an LG3 Scion Scientific Frame grabber and NIH Image software. A Sony CCD video camera (model AVC-D7) mounted on an Olympus CK2 compound microscope was connected to the computer so that magnified images could be analyzed directly after calibration with a stage micrometer. Each corneal slide preparation was viewed at 40X magnification in the approximate middle of nine different eye regions (Fig. 1). Every image was made with thresholding activated in order to produce a binary

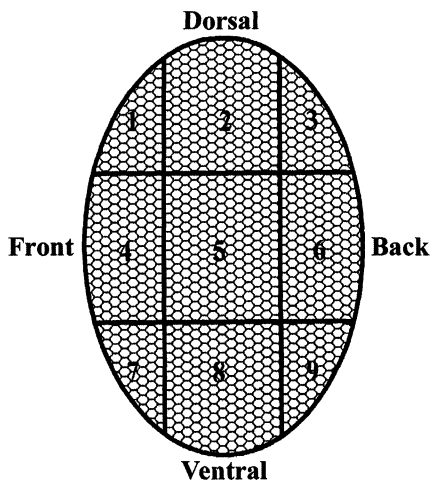


Fig. 1. Nine regions of the eye chosen for sampling of facet areas.

image for automatic measurements. They were then adjusted in focus and contrast to produce the clearest possible borders between facets. The auto-measure tool was used to trace the perimeter and calculate the area of 10 randomly chosen facets in each eye region, so that a total of 90 facet area measurements were made for each eye that were used to calculate the mean facet area for each region in each individual. The means for each region reported in Table 1 were calculated from these means.

Statistical analysis: All area measures and counts of facets (which are correlated with area) were square-root transformed to a single dimension before analyzing their correlations with linear measures of body size. Statistical testing of correlations and comparisons was done using SYSTAT (Version 5.02 for Windows) at the 0.05 level of significance. Summary statistics are given as mean \pm standard error of the mean.

RESULTS

Eye surface area, facet number, and facet size

Females of *A. leilia* are significantly larger than males (forewing length: males, 21.7 ± 0.19 mm ($n = 21$); females, 25.5 ± 0.39 mm ($n = 17$); $t = 8.68$; $df = 23$ (assuming unequal variances); $p < 0.0001$).

Table 1. Variation in facet area among the nine regions in males and females. Facet area is given as mean \pm SEM. See text and Fig. 1 for definitions of regions.

Region	Facet Area (μm^2)		
	Males (n = 16)	Females (n = 15)	% Difference
1	333 \pm 8	264 \pm 11	25.9
2	302 \pm 8	251 \pm 11	20.1
3	312 \pm 8	287 \pm 12	8.5
4	427 \pm 11	333 \pm 12	28.3
5	386 \pm 11	308 \pm 12	25.5
6	353 \pm 12	304 \pm 11	16.2
7	332 \pm 12	276 \pm 8	20.1
8	309 \pm 10	260 \pm 8	18.9
9	293 \pm 8	262 \pm 10	11.7

Hence, we controlled for body size in comparisons of eye size both within and among sexes. Eye surface area is significantly positively correlated with forewing length (Fig. 2) in both males ($n = 15$, $F_{1,13} = 13.02$, $p = 0.003$, $r^2 = 0.500$) and females ($n = 13$, $F_{1,11} = 14.25$, $p = 0.003$, $r^2 = 0.564$). The slopes of the regression lines for the two sexes, however, are not significantly different (ANCOVA: $F_{1,24} = 2.15$, $p = 0.156$). When effects of forewing length are controlled, eye surface area is significantly larger for males than females (ANCOVA: $F_{1,25} = 36.3$, $p < 0.001$), so for a given body size, male eyes are about 30% larger in surface area than those of females.

Facet number is significantly positively correlated with forewing length (Fig. 3) in males ($n = 14$, $F_{1,12} = 5.23$, $p < 0.05$, $r^2 = 0.303$) but not females ($n = 12$, $F_{1,10} = 0.023$, $p = 0.881$, $r^2 = 0.002$). Facet number is also significantly positively correlated with eye size (Fig. 4) in both males ($F_{1,12} = 24.72$, $p = 0.003$, $r^2 = 0.673$) and females ($F_{1,12} = 7.217$, $p = 0.0198$, $r^2 = 0.376$). The slopes of the regression lines for the two sexes are not significantly different (ANCOVA: $F_{1,24} = 0.18$, $p = 0.67$). When effects of eye surface area are controlled, females have more facets than males ($F_{1,24} = 11.15$, $p = 0.003$).

Facet size or area is not related to forewing length (Fig. 5) in either sex (males, $F_{1,13} = 0.004$, $p = 0.953$, $r^2 < 0.001$; females, $F_{1,11} = 0.981$, $p = 0.343$, $r^2 = 0.082$). However, males have facets that are significantly larger than those of females (ANOVA: $F_{1,29} = 26.5$, $p < 0.001$). Facet area is not correlated with eye size in either males ($F_{1,13} = 0.402$, $p = 0.537$, $r^2 = 0.030$) or females ($F_{1,13} = 2.502$, $p = 0.138$, $r^2 = 0.161$).

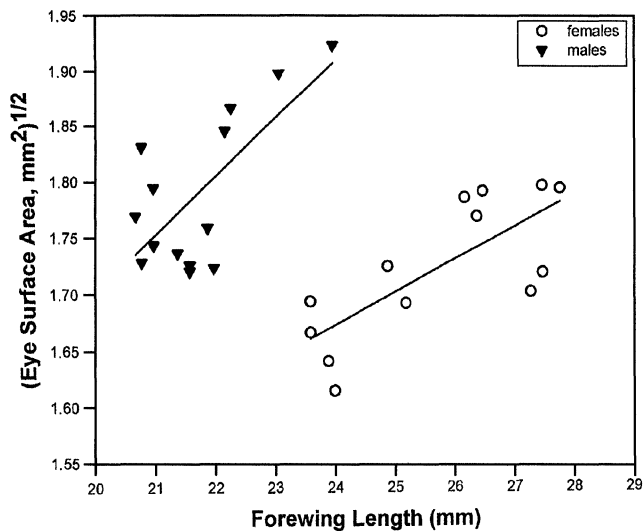


Fig. 2. Relationship between eye size and forewing length in *A. leilia*. Lines represent least squares linear regression for males (▼) and females (O).

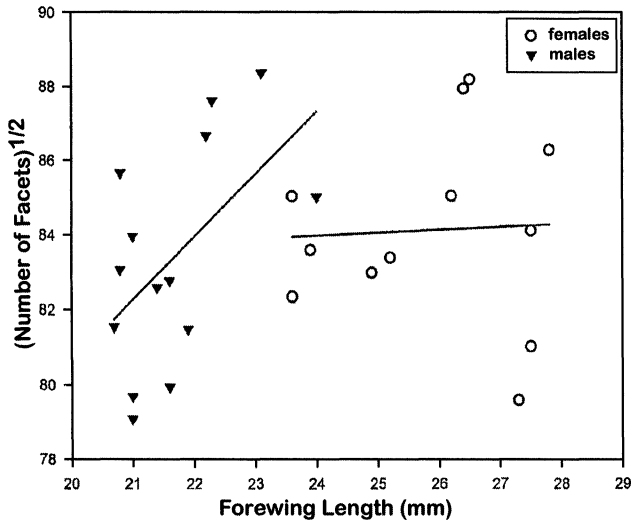


Fig. 3. Relationship between facet number and forewing length for *A. leilia*. Lines represent least squares linear regression for males (▼) and females (O).

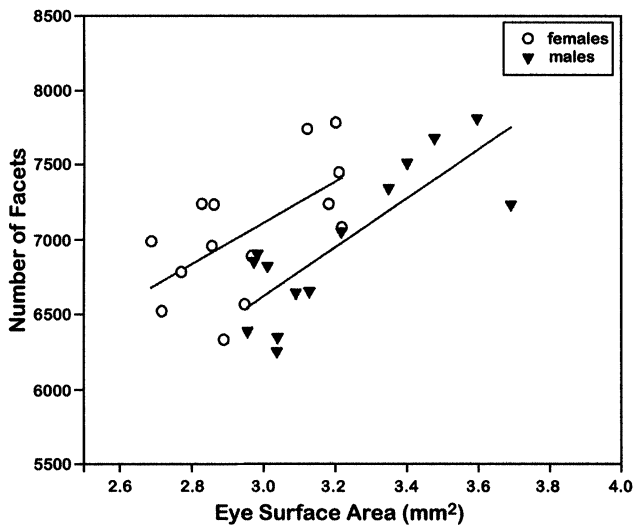


Fig. 4. Relationship between facet number and eye size for *A. leilia*. Lines represent least squares linear regression for males (▼) and females (O).

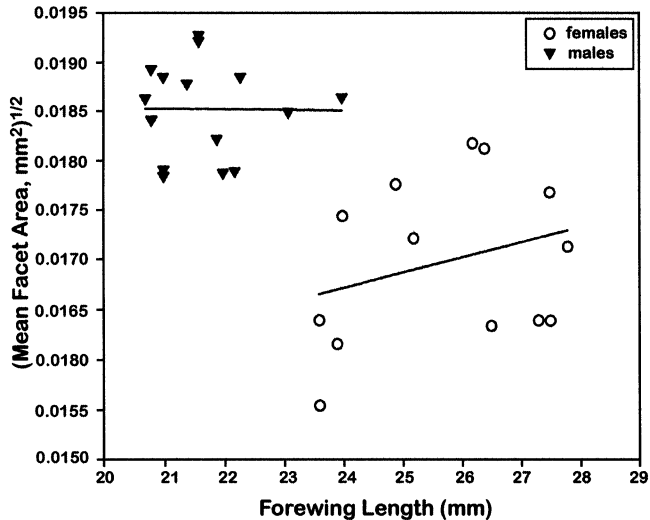


Fig. 5. Relationship between mean facet area and forewing length for *A. leilia*. Lines represent least squares linear regression for males (▼) and females (O).

Regional Variation in Facet Size

Both males and females show a significant effect of eye region on facet size (ANOVA: $p < 0.001$ for both sexes). Males have significantly larger facets than females ($df = 21$, $t = -4.7$ to -2.7 , $p = 0.0001$ to 0.012) in all regions except region 3 which is dorsal and posterior ($t = -1.7$, $p = 0.104$). The proportional difference in facet size between the sexes was largest in regions 1 (dorso-frontal), 4 (midline frontal) and 5 (midline lateral), while all other regions differed in facet area by 20% or less (Table 1).

To examine patterns of change in facet size from front to back and from dorsal to ventral, regions were pooled into horizontal and vertical blocks. First, to examine dorsal to ventral changes, regions 1, 2 and 3 were pooled as a dorsal block; regions 4, 5 and 6 were pooled as an equatorial block; and regions 7, 8 and 9 were pooled as a ventral block. Significant differences in facet area were found between blocks for both males and females (ANOVA: males, $F_{2,42} = 38.8$, $p < 0.001$; females, $F_{2,36} = 8.882$, $p = 0.001$). Post hoc pairwise comparisons of blocks showed that facets in the equatorial block were significantly larger than either dorsal or ventral in both sexes (Tukey's: $p < 0.001$), and no significant difference existed between dorsal and ventral blocks.

Next, to examine front to back changes, regions 1, 4 and 7 were pooled as an anterior block; regions 2, 5 and 8 were pooled as a lateral block; and regions 3, 6 and 9 were pooled as a posterior block. No significant differences were found between these blocks for females (ANOVA: $F_{2,36} = 0.96$, $p = 0.392$), but highly significant differences were found between blocks for males (ANOVA: $F_{2,42} = 13.5$, $p < 0.001$). Tukey's post hoc comparisons for the males showed that the anterior block had significantly larger facets than either the lateral ($p = 0.003$) or the posterior ($p < 0.001$) blocks.

DISCUSSION

Our study of *A. leilia* confirms that males have eyes that are larger in surface area than females. Also, the larger eye surface area in males is due to larger facets rather than more facets. Finally, compared with females, facets in males are especially larger in the dorso-frontal region of the eye. These results suggest that the larger eyes of males are at least regionally more acute than those of females.

The overall differences in eye surface area between the sexes agree with those reported by Yagi and Koyama (1963) who examined 84

species from 25 lepidopteran families and found that the ratio of male eye surface area to that of females ranged from 1.1 to 1.4. In *A. leilia* this ratio is about 1.1. Our results concerning facet number and facet size, however, contradict other generalizations they make concerning butterfly eye morphology. Yagi and Koyama state that the number of facets is always larger in males than in females, that larger eyes have larger facets, and that facet size is generally uniform across the eye of a butterfly. In *A. leilia*, however, although facet number varies with eye size, for a given eye size, male eyes actually have *fewer* facets than female eyes. We found no correlation in *A. leilia* between facet size and eye size, although males have larger facets than females, regardless of their body size or eye size. In *A. leilia* facet size varies among eye regions, and the variation is greater in males than in females (Table 1).

These differences between our results and those of Yagi and Koyama (1963) may be due to differences in the taxonomic placement and behavior of the species studied. Although their study was presented as a broad survey of lepidopteran eye morphology, the data presented regarding sexual differences were limited. For comparison between the sexes in facet number, Yagi and Koyama (1963) noted sexual differences in facet number in three species of butterflies in two families: *Papilio xuthus* (Papilionidae), *Colias erate poliographus* (Pieridae), and *Pieris rapae crucivora* (Pieridae). In contrast, *A. leilia* is in the family Nymphalidae. For comparison between the sexes in facet size, Yagi and Koyama (1963) used only the two pierids.

Also, in the species studied by Yagi and Koyama (1963), males use a different mate locating tactic than *A. leilia* males. Male butterflies display two broad types of mate-locating behavior: perching and patrolling (Scott 1974, 1975, 1982; Rutowski 1991). The former tactic involves sitting and waiting for females to fly into view, while the latter involves actively searching out mates in flight. In the species studied by Yagi and Koyama (1963), the males are patrollers, whereas in *A. leilia* males are perchers (Scott 1986). Males might have different eye specializations depending on the mate locating tactic they employ. Patrollers might benefit most from forward-directed acute zones, and possibly ventral acute zones if the males search for perched females. Perchers, on the other hand, might benefit most from dorsal and/or forward-directed acute zones, depending on where the males perch. Whatever the explanation, discrepancies between Yagi and Koyama's and our results suggest that it is premature to generalize across species about the details of intersexual differences in eye structure in butterflies.

The fact that the increase in facet area is not uniform across the eye and differs between males and females lends support to the idea that mate location is the driving force behind male eye design in *A. leilia*. In *A. leilia* both males and females have larger facets along the equator of the eye than either above or below it. This pattern is common to many nectar-feeding and foliage-eating flying insects, which have been shown to have decreased interommatidial angles along the eye equator (Land 1989, 1990). Males, however, also show a significant increase in facet area in the frontal eye regions as compared to more posterior facets. Such a pattern is not seen in females. Although facet areas are greater in males than in females for all eye regions, they were found to be disproportionately larger in the dorso-frontal regions. This suggests that male eyes have increased resolving power in these regions. In other words, acute zones in males are directed both horizontally and forward/upward.

Intrasexual Patterns of Eye Morphology

Both males and females show a significant positive relationship between body size and eye size (Fig. 2), but the structural changes that accompany increasing eye size with body size are different for each sex. In females, neither facet number nor facet size varies significantly with body size. Given that there is little or no space between facets, however, the increase in eye size with body size must be due to an increase in facet number, size or both. We did not detect either trend perhaps because they are too weak to detect with our sample of 13 females.

Males, on the other hand, do show a significant positive relationship between facet number and body size (Fig. 3), but no relationship between facet size and body size. These findings are perplexing. Males have larger facets than females, especially in regions used for mate location, which is probably due to the need for increased resolution. Why, then, do larger males not have still larger facets? Perhaps increases in facet size and decreases in interommatidial angles could give large males a competitive edge over small males in mate location.

We offer two possible reasons for the limit to male facet size. First, males of *A. leilia* may be physiologically or developmentally constrained at a maximum facet size. Second, further increases in facet size may not increase acuity as much as packing in more facets (with smaller interommatidial angles). It is true that larger facets can have an

advantage over smaller ones by reducing diffraction patterns and increasing resolution when combined with smaller interommatidial angles (Land 1990), but there is a point at which resolution can no longer be improved by increasing facet size, due to other optical limitations (Horridge 1977).

The structure of the butterfly eye has been fine tuned by various selection pressures, and the relationship between eye morphology and behavior is worth further study. It has already been shown that the distribution of visual pigments across the eye can vary between sexes within a species and between closely related species according to demands placed on the visual system for mate identification, territorial behavior, and location of larval food plants (Bernard and Remington 1991). Differences in male mate-locating behavior are correlated with differences in flight muscle structure, wing size and shape, and wing-beat frequency in butterflies (Scott 1982; Wickman 1992). In perching species, males are found to have muscle mass and wing morphology specialized for quick acceleration and speed, while patrollers are designed for flight endurance. Comparative studies of butterfly eye morphology might also reveal differing degrees and types of sexual dimorphism depending on male mate locating tactics as well as female behavior patterns.

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