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The eyes of a patrolling butterfly: Visual field and eye structure in the Orange Sulphur, *Colias eurytheme* (Lepidoptera, Pieridae)

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Abstract

Sensory information plays a critical role in determining an animal's behavior on both proximate and evolutionary timescales. Butterflies, like many other insects, use vision extensively over their lifetimes, and yet relatively little work has been published to date on their visual capabilities. We describe the visual system of a pierid butterfly, *Colias eurytheme*, with the ultimate goal of better understanding its role in shaping the behavior of this animal. We made several measurements: visual field dimensions, eye surface area, interommatidial angle ($\Delta\varphi$), facet diameter (D), and eye parameter (p). *C. eurytheme* had a large visual field and considerable regional variation in visual acuity, as inferred by $\Delta\varphi$ and D. When compared to females, males had larger eye surface areas, smaller $\Delta\varphi$, and larger D in all regions except ventrally. Both sexes had proportionally large eye surface areas compared to other butterflies. Minimum p in males was small, indicating that some regions of their eyes may operate close to the diffraction limit. Finally, we found that both eye surface area and D scaled positively, but with negative allometry to body size. We discuss the relevance of these visual characteristics to the biology and behavior of *C. eurytheme*.
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1. Introduction

Vision and behavior are intimately intertwined at both proximate and evolutionary time scales. At the proximate level, visually guided behavior should reflect the quantity and quality of information that the visual system delivers to the brain. In evolutionary time, selection is expected to produce a tight link between the design of an animal's visual system and the demands of its lifestyle and behavior. Therefore, knowledge about the visual capabilities of animal eyes should inform not only our understanding of the role of vision in shaping interactions with conspecifics and their surroundings, but also our understanding of how selection in different behavioral contexts has influenced the evolution of eyes (Kirschfeld, 1976; Endler, 1992; Hornstein et al., 2000; Egelhaaf and Kern, 2002; Land and Nilsson, 2002).

Butterflies use vision extensively throughout their adult lives (Scott, 1974; Silberglied and Taylor, 1978; Kinoshita et al., 1999; Kelber and Pfaff, 1999; Kemp, 2000; Rutowski et al., 2001). The optics (Nilsson et al., 1984, 1988) and photoreceptors (Shimohigashi and Tominaga, 1991; Hsu et al., 2001; Briscoe et al., 2003) of butterfly eyes are well known. However, less is known about butterfly eye structure and its implications for spatial resolution and visual sensitivity. The eye structure of only one species of butterfly has been described in detail (*Asterocampa leilia*, family Nymphalidae; Ziemba and Rutowski, 2000; Rutowski and Warrant, 2002). Measurements from other species of butterflies have been published (Yagi and Koyama, 1963; Land and Osorio, 1990; Rutowski, 2000; Lund et al., 2001; Stavenga et al., 2001), but no comprehensive description of eye structure is available for any other species of butterfly. Additional work describing the eyes of other species, particularly those in families other than Nymphalidae, is needed to allow a more general understanding of the nature of butterfly vision, and, by extension, their visually based behavior.

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The Orange Sulphur butterfly, *Colias eurytheme* (family Pieridae), is an excellent candidate for this research. The roles of vision and visual signals in the behavior of this species have been studied in detail, especially in the context of mating behavior: males spend much of their lives patrolling their environment for mates, using vision to detect and track females (Rutowski, 1978, 1985; Rutowski et al., 1981; Ellers and Boggs, 2002). Furthermore, males possess a brilliant ultraviolet iridescence that is used in female mate choice (Silberglied and Taylor, 1978; Rutowski et al., 2005). Nevertheless, for this species and for other pierids, little is known about the quality of their vision and how this might influence their behavior.

In this study, our primary goal was to describe eye morphology and structure in *C. eurytheme*, including visual field dimensions, eye surface area, and regional variation in interommatidial angle ($\Delta\varphi$), facet diameter (D), and eye parameter (p). Such measurements provide more information than can be derived from eye size alone (Rutowski, 2000), permitting inferences about visual acuity and sensitivity, how they vary from one part of the visual field to the next, and how they differ between the sexes (Land and Nilsson, 2002). Regions of high visual acuity are typically characterized by small $\Delta\varphi$ and large D, which are required to densely sample a region of space with narrowly focused ommatidia (Land, 1997). Increasing D also increases the photon catch of an ommatidium, resulting in improved sensitivity (Land and Nilsson, 2002). Finally, p is the product of D (in μm) and $\Delta\varphi$ (in radians), and provides an indication of how closely a set of ommatidia operates to the limits imposed by diffraction (Snyder, 1977). In a compound eye in which the resolution limit set by D matches the resolution limit set by $\Delta\varphi$, p should equal approximately 0.29 (Snyder, 1977).

2. Materials and methods

2.1. Collection and handling of animals

We collected butterflies in the summer of 2002 from alfalfa fields located in Chandler (Maricopa County), Arizona. Animals were placed in 35 mm film vials or glassine envelopes and transported to Arizona State University, where they were stored at about 4 °C until they were used. All $\Delta\varphi$ and visual field measurements were conducted within 2 days of capture. Specimens were frozen for later measurements of D and eye surface area.

We used hind femur length as an indicator of body size. We measured it with an ocular micrometer calibrated to the nearest 0.01 mm, which was mounted in the eyepiece of a dissection microscope.

2.2. Surface area measurements

Eye surface area was estimated using the procedure described in Rutowski (2000). Briefly, several linear dimensions of the eye were measured in millimeters with a calibrated micrometer in the ocular of a dissecting microscope. Eye surface area was estimated from these measurements by assuming that the eye was a partial oblate ellipsoid.

2.3. Measurement of visual field

The visual field of 10 individuals from each sex was measured as follows. The head was removed from chilled live individuals and mounted using petroleum jelly onto a small tiltable platform attached to a goniometer stage. This platform allowed the mounted head to be rotated in the yaw plane at known degree intervals independent of the goniometer stage, thereby enabling visual field measurements that extended beyond a single hemisphere. The left eye of each individual was illuminated orthodromically by directing a beam of light onto a glass cover slip positioned directly below the objective of a microscope. This cover slip was positioned at 45° to the optical axis of the microscope (model MZM1, Askania Mikroskop Technik Rathenow), thereby reflecting the beam into the eye along the axis of viewing. This permitted us to visualize the eyeshine, which was used to identify those ommatidia pointed directly at the objective of the microscope (Stavenga, 1979). Each animal's head was oriented such that (1) the back of the head capsule lay flush with the device attached to the goniometer stage, and (2) when the head was viewed from the front and we adjusted the pitch of the head, the visible binocular eyeshine moved in a symmetrical fashion over the surface of the eyes. The reference point of origin for all measurements of latitude and longitude was provided when the head was positioned in this manner and all axes of the goniometer were set at 0°. The anterior and posterior limits of the visual field were determined for each 10° increment of latitude between +70° and −70° by rotating the head in both longitudinal directions until the eyeshine disappeared from the left eye.

To calculate the total visual field of an individual as well as the extent of binocular overlap, we assumed symmetry in the visual field for both eyes. Therefore, the mirror image of measurements obtained from the left eye was used as a surrogate for measurements of the visual field of the right eye. The resulting data were plotted in a two-dimensional graph on which we visually interpolated a line indicating the edges of the visual field of both eyes. For each 10° interval of latitude we used Scion Image (Scion Corp) to calculate the proportion of the visual field seen at that latitude by both or just one eye. These estimates were corrected using a cosine function for the distortion introduced by the two-dimensional plot, and then summed and expressed in units of $\pi \cdot$ steradians.

2.4. Measurements of $\Delta\varphi$

To measure $\Delta\varphi$, we dealated butterflies and immobilized them in 1.7 ml microcentrifuge tubes mounted on a goniometer. We then positioned the goniometer under a

microscope, and illuminated the eye orthodromically to visualize the eyeshine as described above. Spot sampling was used to assess variation in $\Delta\varphi$ around the eye and among individuals. Spot sampling allows one to sample a large number of individuals. This permitted us to use statistics to test for differences among eye regions and across sexes, which has rarely been done in the literature on eye morphology. Furthermore, by replicating measurements in each region, we were able to control for measurement error.

Individuals were sampled for $\Delta\varphi$ twice in each of five different regions of the eye, which were chosen to provide a general representation of regional variation in acuity around the animal's visual field (Ziemba and Rutowski, 2000; Rutowski and Warrant, 2002). The regions were (Fig. 1): dorsal (facets adjacent to the insertion of the antennae), anterior (facets located just above the clypeolabral suture on the anterior edge of the eye), anterioventral (facets next to the insertion of the proboscis), ventral (facets located near the ventral-most edge of the eye), and lateral (facets along the approximate equator of the eye, $90°$ from the front of the eye). The head was positioned such that when the head was rotated, the pseudopupil moved along the vertical facet rows. We did not measure along the horizontal or diagonal axes because (a) previous work on butterfly eyes indicates they are essentially spherical and that $\Delta\varphi$ does not vary substantially along different axes (Rutowski and Warrant, 2002) as it does, for example, in hymenopterans (Zollikofer et al., 1995), and (b) we wanted to maximize the number of regions and individuals we sampled. In each region of the eye, we

Fig. 1. A side view of the head of a butterfly showing the eye regions sampled for $\Delta\varphi$ and D. Drawing modified from that by Barbara Terkanian in Rutowski (2000).

measured the angular change required to move the eyeshine across six facets. We then used this angle to calculate the average $\Delta\varphi$ in that region of the eye.

2.5. Measurements of D

We measured D on the same individuals for which we measured $\Delta\varphi$. We captured images of each eye region (see above) with a video capture computer card (ATI Radeon All-In-Wonder) that received input from a video camera mounted on the microscope. The images were analyzed using ImageTool (University of Texas Health Science Center). For each image we measured to the nearest $0.01\,\mu m$ the length of a vertical row of five facets, from which we calculated the mean D. Measurement software was calibrated using a same-magnification image of a scale accurate to $10\,\mu m$. We made two measurements along different facet rows in each region.

2.6. Statistics

All statistical tests were conducted at the 0.05 level of significance, and we report central tendency as mean $\pm$ standard error. We used SAS software (SAS Institute, Inc.) for our analyses. Eye surface area data were analyzed using ANCOVA with sex included as a factor and hind femur length included as a covariate. Assumptions of normality and equal variance were verified. In this analysis, eye surface area estimates were square root transformed to reduce them to one dimension. In addition, we log transformed the square root of eye surface area and hind femur length in order to linearize the relationships and to allow the calculation of allometric exponents.

We examined overall visual field dimensions for differences between the sexes using ANCOVA with sex as a factor and hind femur length as a continuous covariate. However, because the total visual field does not indicate the extent of binocular vision within the visual field, we tested for differences in binocular vision across sexes using a second ANCOVA with sex as a factor and hind femur length as a continuous covariate.

Patterns of variation in $\Delta\varphi$, D, and p among regions and individuals were each evaluated using mixed model ANOVAs. Sex, eye region, individual (a random variable nested within sex) and their interactions were included as factors, with hind femur length added as a covariate. Continuous variables were again log transformed to allow estimation of allometric exponents. Assumptions regarding heterogeneity of variance and normality were verified. Non-significant interaction terms were removed from the model. Tukey–Kramer post hoc tests were conducted to identify significant differences between sexes and regions. In those cases in which significant interactions between hind femur length and region were detected, we conducted separate ANCOVAs within each region to identify the cause of the interaction. When assessing the effects of hind femur length in each of these ANCOVAs, we adjusted our
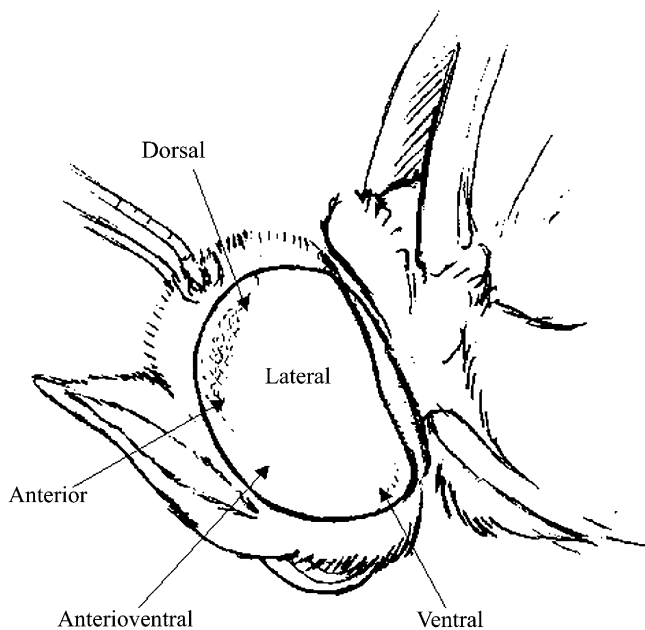
experimentwise error rate α using the Bonferroni method (Sokal and Rohlf, 1995).

3. Results

3.1. Eye surface area

Males had significantly larger eye surface areas ($3.71 \pm 0.09\,mm^2$) than females ($2.96 \pm 0.06\,mm^2$; $F_{1,18} = 49.67$, $P < 0.0001$; Fig. 2). This was true even if hind femur length was removed from the model. Therefore, despite their smaller body size, males had larger eyes than females. Under isometry, hind femur length and square root-transformed eye surface area were expected to scale with an allometric exponent of 1. However, we found that the allometric exponent (a) was significantly lower (95% confidence interval of slope: $-0.04 \leqslant a \leqslant 0.44$; Table 1), indicating that eye surface area scales with negative allometry to body size.

3.2. Visual field shape and dimensions

The visual field of *C. eurytheme* is large, encompassing 93% of the spherical space around the butterflies, with only a small blind spot behind the head not seen by either eye (males: Fig. 3; females: Fig. 4). Nevertheless, their vision is mostly monocular; only a small, mostly frontal region of the visual field is seen simultaneously by both eyes (4.5%). The sexes do not differ significantly in the size of the total visual field (females: 3.782 ± 0.031 π ster; males:

Table 1
Allometric exponents (a) estimated as the slopes of log–log plots of each variable on hind femur length

Variable	95% C.I. around allometric exponents
$\sqrt{}$Eye surface area	$-0.04 \leqslant a \leqslant 0.44$
$\Delta\varphi$**	
Dorsal	$-2.08 \leqslant a \leqslant -0.24$
Anterior	$-0.62 \leqslant a \leqslant 1.31$
Lateral	$-0.99 \leqslant a \leqslant 0.86$
Ventral	$-2.97 \leqslant a \leqslant -0.06$
Anterioventral	$-2.23 \leqslant a \leqslant 0.66$
D	$0.20 \leqslant a \leqslant 0.54$
p**	
Dorsal	$-1.81 \leqslant a \leqslant 0.11$
Anterior	$-0.21 \leqslant a \leqslant 1.86$
Lateral	$-0.31 \leqslant a \leqslant 1.33$
Ventral	$-2.75 \leqslant a \leqslant 0.27$
Anterioventral	$-1.41 \leqslant a \leqslant 0.34$

Estimates of coefficients are shown as 95% confidence intervals to allow comparison of the estimates to expected allometric exponents under isometry.
**Allometric exponents for $\Delta\varphi$ and p were calculated for each eye region because of significant region∗body size interactions.

3.790 ± 0.40 π ster; $P = 0.852$). In addition, sexes do not differ significantly in the extent of binocular overlap (females: 0.187 ± 0.024 π ster, males: 0.175 ± 0.013 π ster, $P = 0.754$).

3.3. Variation in $\Delta\varphi$

The ANOVA conducted on the $\Delta\varphi$ data (Table 2) showed that the effects of both sex and region were significant. However, the sex∗region interaction term was also significant ($F_{4177} = 4.03$, $P = 0.0037$), because the magnitude of differences between sexes varied among the eye regions (Fig. 5). Males had significantly smaller $\Delta\varphi$ values than females in all regions except ventrally, where the two sexes were equivalent (Tukey–Kramer post hoc test, $t = 1.87$ $P = 0.8395$). In males, the smallest $\Delta\varphi$ (indicating relatively high visual acuity) was in the frontal eye regions ($0.85°$ and $0.72°$ in anterior and anterioventral regions, respectively). Male $\Delta\varphi$ were larger in the ventral ($1.09°$) and lateral regions ($1.26°$), and still larger in the dorsal region ($1.84°$). Females followed a similar pattern, except that anterior, anterioventral, and ventral $\Delta\varphi$ were all equivalent ($1.24°$, $1.18°$, and $1.23°$, respectively).

The hind femur length∗region interaction term was also significant ($F_{4177} = 3.30$, $P = 0.0124$). To probe this relationship, we conducted ANCOVAs on each region individually with sex and hind femur length included as factors (Table 1). In the dorsal region, there was a significant relationship between $\Delta\varphi$ and hind femur length ($P = 0.02$, scaling coefficient $= -1.16$); however, this relationship was not significant in other regions. In no
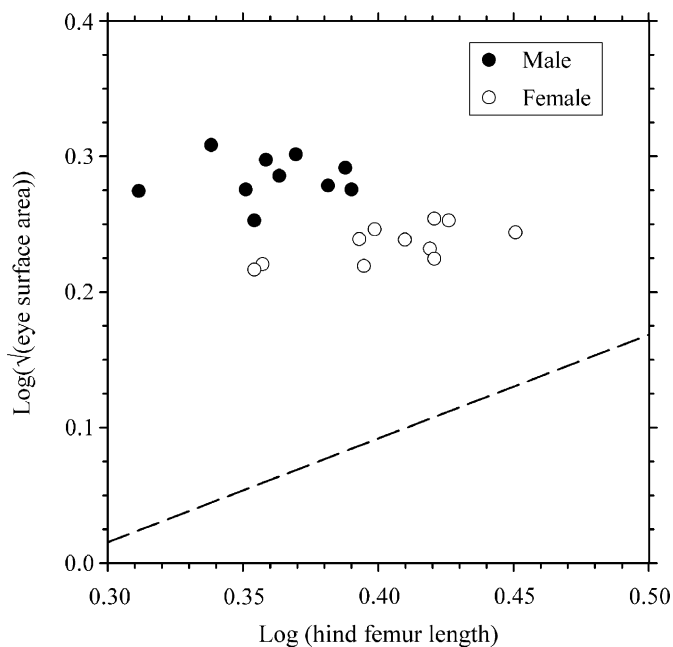

Fig. 2. Log–log plot comparing eye surface area and hind femur length for *C. eurytheme* males and females. The dashed line represents the least-squares regression line from Rutowski (2000) describing the relationship between eye surface area and hind femur length for 16 species of butterflies.
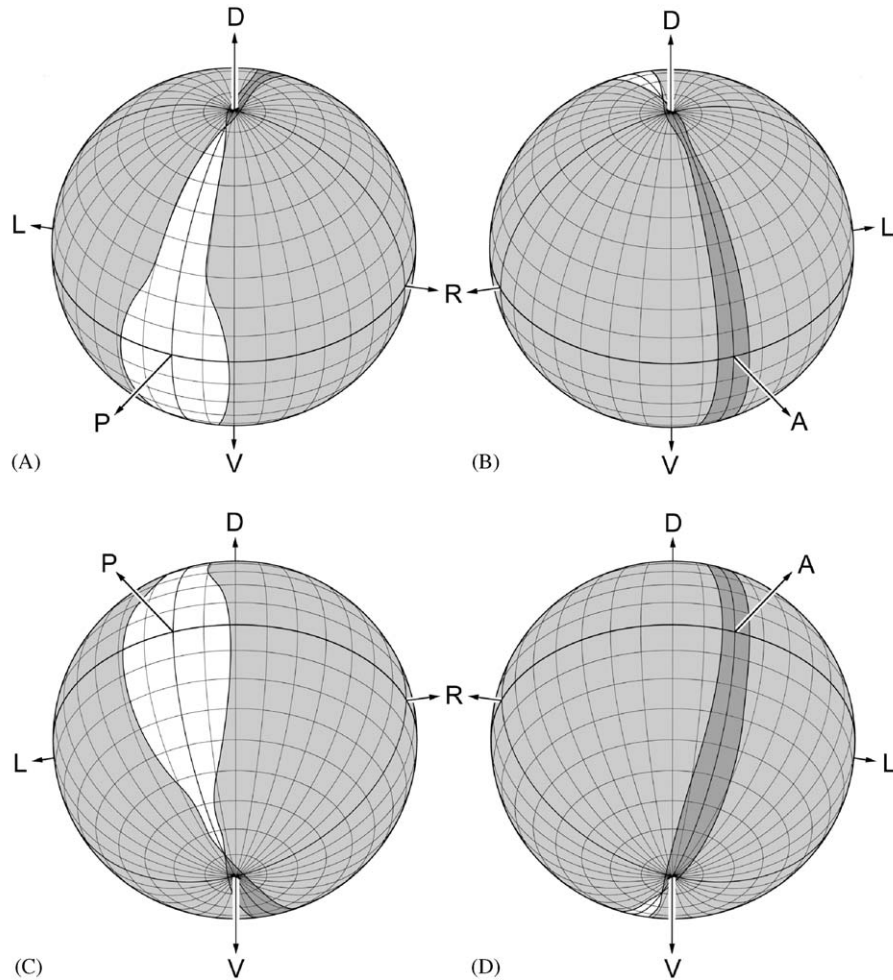
Fig. 3. Visual field of *C. eurytheme* males shown from four perspectives: (A) dorso-posterior view, (B) dorso-anterior view, (C) ventro-posterior view and (D) ventro-anterior view. The white area is that part of the space around the butterfly that is not detected by either eye. The light shading is the region of monocular vision, while the darker shading is where vision is binocular. Abbreviations: D, dorsal; V, ventral; A, anterior; P, posterior; R, right; L, left.

region was there a significant relationship between $\Delta\varphi$ and hind femur length using a Bonferroni-corrected α of 0.01.

3.4. Variation in D

As expected, facet diameter, like $\Delta\varphi$, varied regionally (Table 3). The sex∗region interaction was highly significant ($F_{4181} = 7.97$, $P < 0.0001$; Fig. 6). Males had significantly larger D than females in all eye regions except ventral (Tukey–Kramer test, $t = 1.54$, $P = 0.87$), and the largest D in males were found in the equatorial regions of the eye (25.8, 26.1, and 26.5 μm in the anterior, anterioventral, and lateral regions, respectively), while those in the ventral (22.6 μm) and dorsal (21.3 μm) regions were smaller. In females, D followed a similar pattern, except that ventral D (21.8 μm) were equivalent to those in equatorial region (22.2 μm in both anterior and lateral regions). Dorsal D measurements in females were the smallest recorded in either sex (18.5 μm).

Facet diameter covaried with body size ($F_{1,18} = 18.24$, $P = 0.0005$). Under isometry, facet diameter would scale to

the first power with body size. However, we found that the allometric exponent estimates were significantly less than one (Table 1), indicating that facet diameter, like eye surface area, scales with negative allometry to body size.

3.5. Eye parameter, p

As with the $\Delta\varphi$ and D data, p varied both among the sexes and among regions (Table 4, Fig. 7). The calculated values of p were significantly smaller in males than in females ($F_{1,18} = 23.06$, $P = 0.0001$) and were smaller in the anterior regions (anterior and anterioventral) than elsewhere. Lateral p's were larger, and dorsal p's were the highest of any region. The sex∗region interaction was not significant ($F_{4,72} = 2.37$, $P = 0.06$).

The hind femur length∗region interaction term was significant ($F_{4,72} = 2.65$, $P = 0.0399$), as was seen in $\Delta\varphi$. However, hind femur length was not a significant term in any of the subsequent ANCOVAs conducted on each individual region using $\alpha = 0.05$ (Table 1).
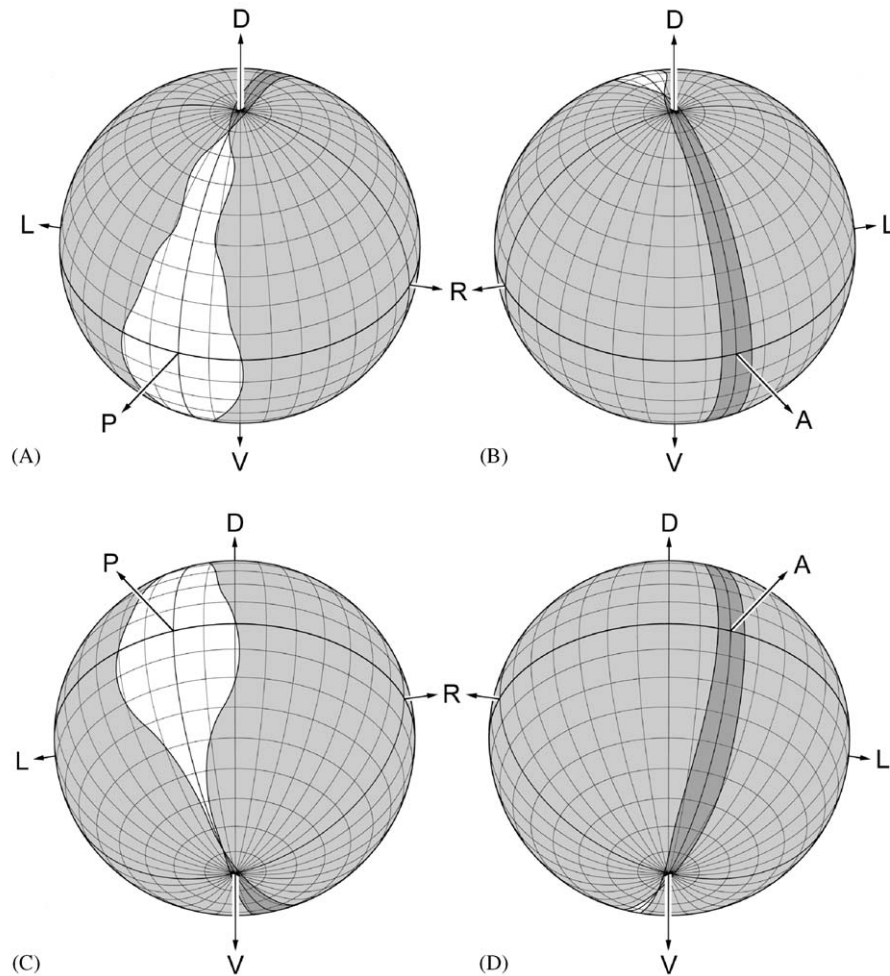
Fig. 4. Visual field of *C. eurytheme* females shown from 4 perspectives: (A) dorso-posterior view, (B) dorso-anterior view, (C) ventro-posterior view and (D) ventro-anterior view. Shading and abbreviations are as described in the caption for Fig. 3.

Table 2
Analysis of variance table for $\Delta\varphi$

Effect	F-value	P-value
Sex	$F_{1,18} = 74.51$	<0.0001
Individual (sex)	$F_{18,177} = 1.52$	0.0865
Region	$F_{4,177} = 5.00$	0.0008
Sex*Region	$F_{4,177} = 4.03$	0.0037
Hind femur length	$F_{1,18} = 7.39$	0.0141
Hind femur length*Region	$F_{4,177} = 3.30$	0.0124

P-values indicate the result of type-III tests for each factor in the model. The analysis was conducted on log-transformed $\Delta\varphi$ and log-transformed hind femur length.

4. Discussion

The results reported here document several general features of eye structure for *C. eurytheme* that are consistent with those reported in other butterflies. The visual field of both males and females is very large and mostly monocular in this species. Visual field dimensions have been published for only one other butterfly, the nymphalid *A. leilia*, (Rutowski and Warrant, 2002), and measured for a few other nymphalids (R. Rutowski, L. Gislen, and E. Warrant, unpublished data). These other species show the same general patterns of visual field size and shape. Also, the highest spatial resolution, as inferred by measurements of $\Delta\varphi$ and D, exists in the anterior region of the eyes of *C. eurytheme*. Again, this is a pattern that appears to be consistent in butterflies and many other insects, and indicates that their best vision is usually located in the anterior region of their eyes (Land, 1997; but see Horridge and McLean, 1978; Labhart and Nilsson, 1995; and Lund et al., 2001 for some exceptions).

Several features of the eyes of *C. eurytheme* were notably different from those found in other butterflies. First, relative eye surface area was quite large compared to measurements from other butterflies (Fig. 2; Rutowski, 2000). The ratio of eye surface area to hind femur length was much larger than in any species measured to date. For *C. eurytheme* males, the 95% confidence interval for this ratio was $1.53 \leqslant \bar{x} \leqslant 1.71$, while the largest ratio reported by
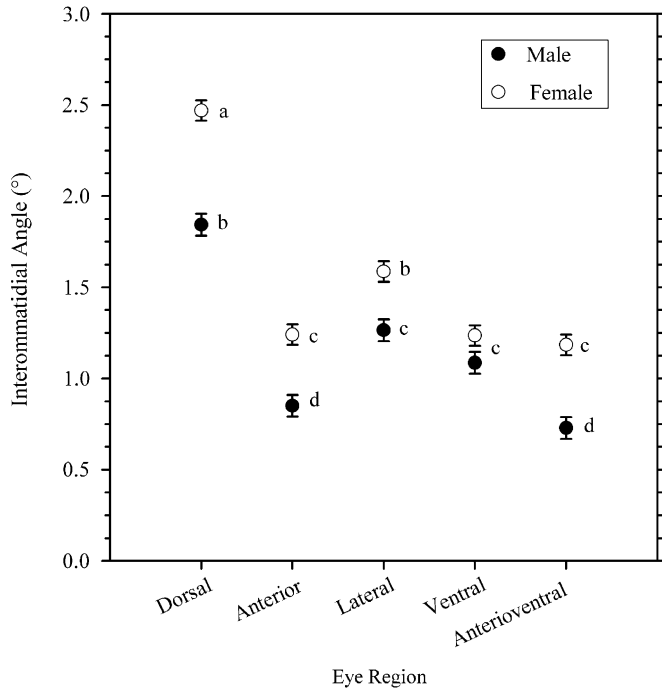
Fig. 5. Regional and sexual differences in $\Delta\varphi$: Regions that share letters are not significantly different from one another, based on Tukey–Kramer post hoc comparison tests. Error bars are $\pm$ standard error. See Fig. 1 for details on eye regions.
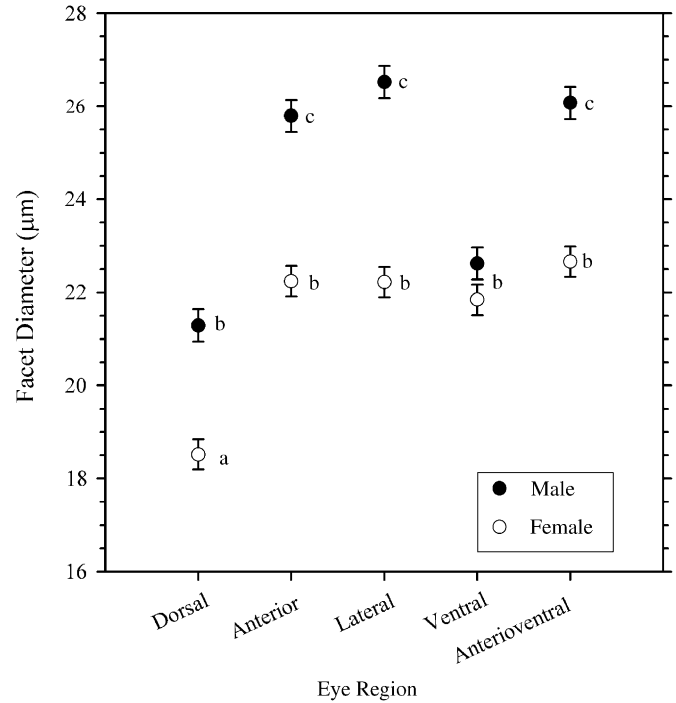

Fig. 6. Regional variation in D: Regions that share letters were not significantly different from one another, based on Tukey–Kramer post hoc comparison tests. Error bars are $\pm$ standard error. See Fig. 1 for details on eye regions.

Table 3
Analysis of variance table for D

Effect	F-value	P-value
Sex	$F_{1,18} = 91.67$	<0.0001
Individual (sex)	$F_{18,181} = 1.34$	0.1689
Region	$F_{4,181} = 78.92$	<0.0001
Sex*Region	$F_{4,181} = 7.97$	<0.0001
Hind femur length	$F_{1,18} = 18.24$	0.0005

P-values indicate the outcome of type-III tests for each factor in the model. The analysis was conducted on log-transformed D, log forewing length, and log hind femur length.

Table 4
Analysis of variance table for p

Effect	F-value	P-value
Sex	$F_{1,18} = 23.06$	0.0001
Individual (sex)	$F_{18,72} = 0.88$	0.5978
Region	$F_{4,72} = 3.12$	0.0201
Sex*Region	$F_{4,72} = 2.37$	0.0604
Hind femur length	$F_{1,18} = 1.13$	0.2919
Hind femur length*Region	$F_{4,72} = 2.65$	0.0399

P-values indicate the result of type-III tests for each factor in the model. The analysis was run on log-transformed p and log hind femur length.

Rutowski (2000) was for *Papilio rutulus* at 1.24. Similarly, for female *C. eurytheme*, the 95% confidence interval for this ratio was $1.13 \leqslant \bar{x} \leqslant 1.21$, while the largest ratio from Rutowski (2000) was found among *P. rutulus* females at 1.03. It is unclear why *C. eurytheme* has such large eyes for its femur length, or when in the history of this lineage proportionally large eyes evolved. Direct comparisons to studies of eye surface area in other pierids cannot be made at this time, because (a) other studies reporting pierid eye surface area used different measures of body size (Yagi and Koyama, 1963; Lund et al., 2001) and (b) Rutowski's (2000) study did not include pierids.

Second, while visual acuity in *C. eurytheme* was greatest in the anterior region, ventral acuity was also quite high, as indicated by low $\Delta\varphi$ and high D. This was most pronounced among females, where ventral visual acuity

was equivalent to that found in the anterior region of the eye. In contrast, ventral visual acuity as measured in both sexes of *A. leilia* was poor compared to anterior acuity: $\Delta\varphi$ was larger (Rutowski and Warrant, 2002) and D was smaller (Ziemba and Rutowski, 2000). A possible adaptive explanation for the high ventral acuity of *C. eurytheme* is that it may enhance perception of male ultraviolet signals (Silberglied and Taylor, 1978; Rutowski et al., 2005). These iridescent signals are reflected via constructive interference off the dorsal wing surfaces (Silberglied and Taylor, 1978), which are most visible from above and slightly behind male conspecifics (R. Rutowski, unpublished data).

Eye surface area and facet diameter scale with negative allometry to body size in *C. eurytheme*. This finding corresponds well with studies of interspecific allometry in other butterflies (Yagi and Koyama, 1963; Rutowski,
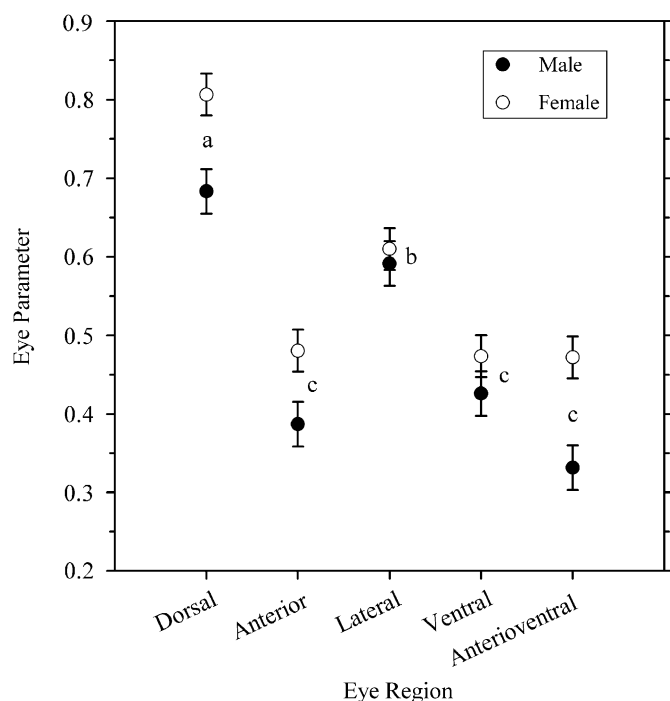
Fig. 7. Regional and sexual differences in *p*: Regions that share letters were not significantly different from one another, based on Tukey–Kramer post-hoc comparison tests. The sex*region interaction was not significant, so all post-hoc comparisons were made between region averages across sexes. Error bars are ±standard error. See Fig. 1 for details on eye regions.

2000), bees (Jander and Jander, 2002), and other insects (reviewed by Wehner, 1981), as well as intraspecific allometry in ants (Zollikofer et al., 1995). These data indicate that, within clades, smaller insects have proportionally larger surface areas and facet diameters than larger individuals. Therefore, smaller insects may have proportionally better vision for their size. There are several possible explanations for this finding. First, while larger eye size should allow superior vision (Kirschfeld, 1976; Land, 1997), eyes are metabolically costly organs (Laughlin et al., 1998; Laughlin, 2001). Therefore, they may be limited in size by metabolic rate, which scales with negative allometry to body size (allometric exponent ≈ 0.75; Hulbert and Else, 2000; West et al., 1997). Furthermore, diffraction increasingly limits minimum acceptance angle as facet diameter decreases (Kirschfeld, 1976; Land, 1997). Therefore, selection favoring large facet diameter, and thus large eye size, should be strongest in smaller insects, despite the higher resource cost (Laughlin, 2001). Consequently, selection may favor the construction of proportionally larger eyes in smaller insects, which would result in eye size scaling with negative allometry to body size.

In *C. eurytheme*, *p* was lowest in the anterioventral region of the eyes of males ($p = 0.33 \pm 0.03$). In an eye in which the resolution limit set by $\Delta\varphi$ matches the resolution limit set by *D*, *p* is expected to equal $\lambda/\sqrt{3}$ (predicted value with abundant light, zero angular velocity, in an eye with ommatidia arranged in a hexagonal lattice; Snyder, 1977).

C. eurytheme has photoreceptors that are sensitive to light from roughly 300 to 700 nm (Post and Goldsmith, 1969), and thus predicted minimum values of *p* range from 0.20 to 0.40. Therefore, the anterior and ventral regions of the eyes of *C. eurytheme* males appear to be operating very close to the limits imposed by diffraction (Fig. 7). Females, on the other hand, had larger *p* estimates. This indicates that the resolution limit of their eyes appears to be set not by *D*, but rather by their relatively large $\Delta\varphi$.

The results of this study provide a glimpse into the visual world of a pierid butterfly. *C. eurytheme* has good vision compared to other butterflies studied to date, particularly in the anterior and ventral regions of their eyes. This is consistent with the behavior of the adults of this species, which use vision to search for mates, oviposition sites, and nectar sources (Silberglied and Taylor, 1978). With this information in hand, we can now seek more direct links between the visual capabilities of these animals and their behavior. For example, upon encountering a female, does a male position himself below her so that he can flash his ultraviolet signals into the acute ventral regions of her eyes? Our data also drive evolutionary questions. In particular, what selective pressures led to the evolution of large relative eye size in these butterflies, and at what point in their lineage did this occur? Our future work will seek to answer these questions by gaining more information about interactions between behavior, visual signals, and vision in *C. eurytheme*, as well as documenting variation in eye structure in additional butterfly species.

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