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Body Size, Rather Than Male Eye Allometry, Explains Chrysomya megacephala (Diptera: Calliphoridae) Activity in Low Light

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RESEARCH

Body Size, Rather Than Male Eye Allometry, Explains *Chrysomya megacephala* (Diptera: Calliphoridae) Activity in Low Light

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ABSTRACT. Male *Chrysomya megacephala* (F.) blow fly compound eyes contain an unusual area of enlarged dorsal facets believed to allow for increased light capture. This region is absent in females and has been hypothesized to aid in mate tracking in low light conditions or at greater distances. Many traits used in the attraction and capture of mates are allometric, growing at different rates relative to body size. Previous reports concerning *C. megacephala* eye properties did not include measurements of body size, making the relationship between the specialized eye region and body size unclear. We examined different morphological features of the eye among individuals of varying sizes. We found total eye size scaled proportionately to body size, but the number of enlarged dorsal facets increased as body size increased. This demonstrated that larger males have an eye that is morphologically different than smaller males. On the basis of external morphology, we hypothesized that since larger males have larger and a greater number of dorsally enlarged facets, and these facets are believed to allow for increased light capture, larger males would be active in lower light levels than smaller males and females of equal size. In a laboratory setting, larger males were observed to become active earlier in the morning than smaller males, although they did not remain active later in the evening. However, females followed the same pattern at similar light levels suggesting that overall body size rather than specialized male eye morphology is responsible for increased activity under low light conditions.

Key Words: sexual dimorphism, visual ecology, behavior, blow fly, allometry

Traits that aid in the capture or attraction of mates can often grow disproportionately in relation to body size among holometabolous insects (reviewed in Emlen and Nijhout 2000). The size of these traits is generally nutrition dependent, as the exaggerated features are not necessary to complete development and can be notably absent in smaller individuals (Emlen 1994). Since the adult size of holometabolous insects is determined by larval feeding (Shingleton et al. 2007), to develop a larger trait as an adult, a larva must feed beyond the minimum requirement to complete metamorphosis (Emlen et al. 2007). This presents a life history trade off in that achieving larger size requires individuals to remain for longer in what may be a relatively vulnerable larval stage (Hanski 1987).

Blow flies are examples of holometabolous insects whose adult size is dependent on larval feeding. Many blow flies feed on carrion (Norris 1965, Hanski 1987, Erzinçlioglu 1996). The use of such a temporary and finite food source leads to the possibility that individuals will not be able to feed long enough to complete development due to intense competition that results in the loss of the food source (Norris 1965, Hanski 1987). Furthermore, blow fly larvae are relatively defenseless against direct predation (Faria et al. 1999) and vertebrate scavenging (Reeves 2009). Once a blow fly has obtained enough nutrition, it can move off the food source, pupate, and become a relatively small adult, or stay on the food and continue feeding, risking predation while possibly becoming a larger adult. A size increase that elevates the performance of a trait used to find mates might generate selection to stay on a food source for longer.

An example of a sexually dimorphic trait that has been hypothesized to help locate mates in a blow fly is the male compound eye of *Chrysomya megacephala* (F.). Adult males possess a dorsal area of ommatidia that are drastically larger than the facets in the ventral region (Kurahashi 1982; Fig. 1a). Females of the same species do not have this area of dorsal enlargement (Sukontason et al. 2008; Fig. 1a). While a compound insect eye containing distinct regions of facet size is not uncommon (reviewed in Land 1997), it is very rare in muscomorph flies, and even more unusual is that the increase in facet size is not accompanied by a change in resolution (van Hateren et al. 1989). This has led to the dorsal area being referred to as the "bright zone" since the enlarged facets are believed to be used for increased light capture, perhaps allowing males to either search for females at lower light levels or from greater distances in higher light levels (van Hateren et al. 1989).

Previous morphological publications concerning the *C. megacephala* male eye (van Hateren et al. 1989, Stavenga et al. 1990, Sukontason et al. 2008) did not investigate the relationship between body size and eye shape. Our first objective was to determine how eye morphology scales with body size in male *C. megacephala*. Our second objective was to analyze the behavioral implications of any variation in eye morphology to gain insight from a visual perspective as to why a blow fly might prolong the risky larval phase of its life cycle to increase its adult body size.

Materials and Methods

Study Groups for Eye Morphology. *C. megacephala* with four sampling histories were used: 1) adult flies caught at a decayed meat bait near the Florida International University campus (referred to as "FIU wild"; 2) adult flies collected in a similar manner in the Florida Keys ("Keys wild"); 3) the adult offspring from a single egg clutch obtained from a laboratory colony originating from Florida International University ("FIU colony"); and 4) the adult offspring from a single egg clutch from a single female captured in Marathon, FL ("Marathon female"). The wild caught flies were all placed in laboratory cages and provided water and sugar for a period of 4 d after capture. At the end of the 4-d period, the flies were killed by freezing. From the two groups of the single egg clutches, some larvae were manipulated to generate a range of adult sizes. To generate small flies, between 25 and 30 larvae from each group were removed from the meat during the early third-instar period and placed directly into sawdust where they could pupate.

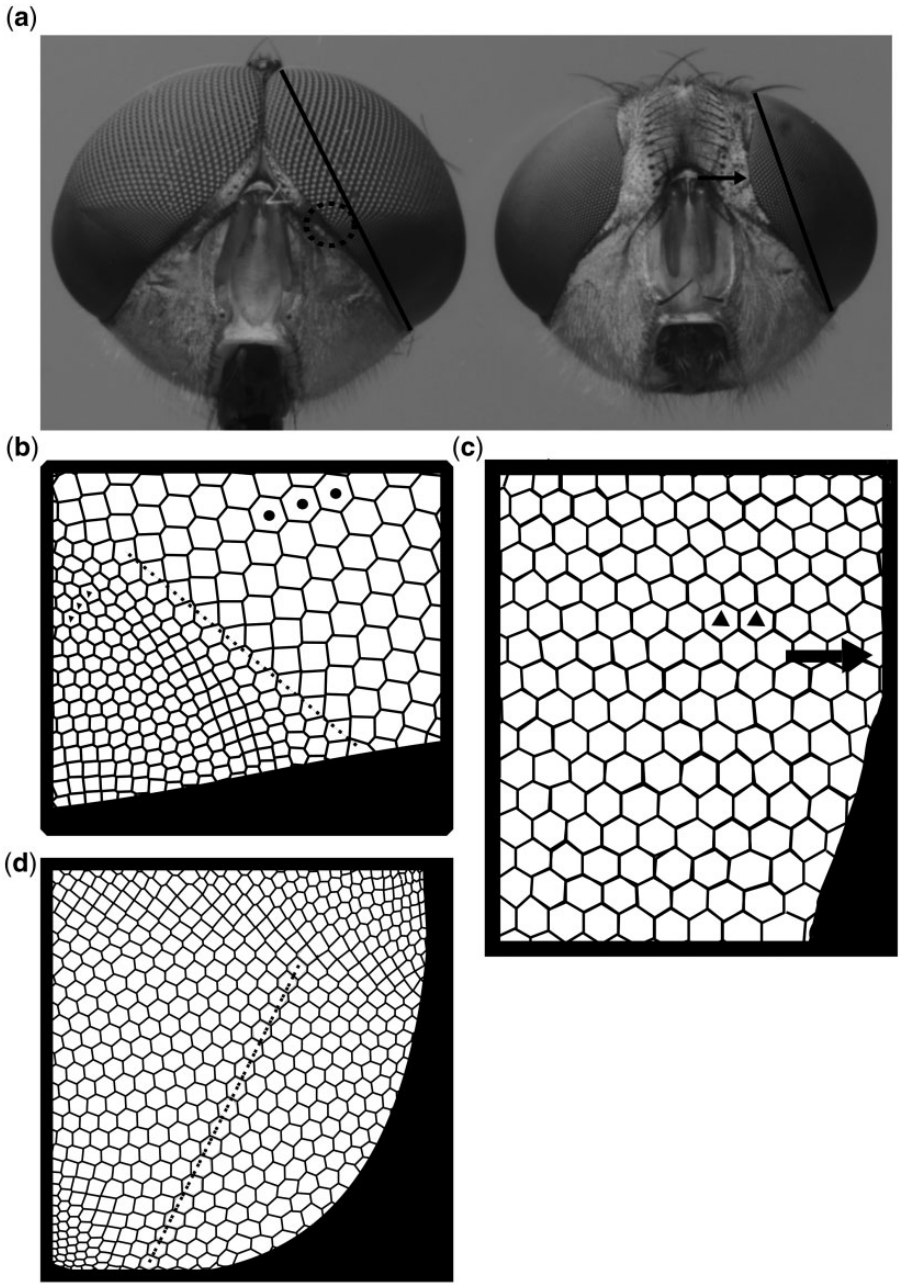

Fig. 1. The compound eye areas morphological measurements were taken from in *C. megacephala*. (a) Photo of male (left) and female (right) heads from *C. megacephala*. The black dotted circle on the male eye is the area shown in (b). The black arrow on the female eye points to area shown in (c). The black lines show the distance measured to determine eye size. (b) Tracing of a male eye replica from the area inside the circle in Fig. 1a. The row used to determine the central facet from which the dorsal and ventral ommatidia were measured is marked with a dashed line. The ventral facets measured are indicated by the three small triangles. The dorsal facets measured are indicated by the three small circles. (c) Tracing of a female eye replica. The two triangles represent the two facets that were measured. The arrow is similar in placement to (a). (d) Tracing of a male eye replica. Ommatidia were counted to indicate the height of the enlarged area. The dashed line indicates the consecutive rows that were counted.

The remaining larvae were provided with chicken liver until each ceased feeding on its own and then pupated in sawdust. Following pupation, the newly emerged adults were allowed 4 d to fully mature before being killed by freezing.

Adult males from all four groups were pinned and assigned a specimen number. We generated a random permutation of the specimen numbers and made measurements of the first 30 specimens on that list. If less than 30 individuals were present, then all individuals were used. If damaged, individuals were omitted from that measurement. As a control for body size independent of male eye morphology, we also pinned adult females from the single egg clutch obtained from Marathon, FL. We chose 20 females in the same manner as the males.

Measuring Fly Size and Eye Morphology. Crossvein length (dm-cu) was used as an indicator of body size (Ireland and Turner 2006). We mounted wings on microscope slides and photographed them at ×25 magnification. Measurements were taken by analyzing photos in Image J (version 1.47; National Institutes of Health; Bethesda, MD). The suitability of crossvein length as an indicator of body size was determined by measuring thorax length (Jander and Jander 2002, Kelber et al. 2006) using a caliper in a subset of males from all four groups. Linear

regression was used to characterize the relationship between thorax length and crossvein length.

Eye size was defined as the distance from top of the medial area of the eye to the bottom of the distal area (black lines in Fig. 1a). Eyes were photographed at ×60 magnification, and the images were viewed and measured using Image J software.

To measure various aspects of eye morphology, casts were made of the eyes using clear nail polish and flattened onto a slide as described in Ribi et al. (1989). We viewed the slides under ×100 magnification and photographed the areas of interest. The photos were viewed and measured using Image J software.

We designated morphological references points to define the facets that were measured across different individuals (Fig. 1b and c). For males, one morphological landmark was the boundary separating the enlarged dorsal ommatidia from the smaller ventral ommatidia. This boundary consists of two rows containing predominantly nonhexagonal shaped ommatidia near the medial portion of the face. Within the ventral most of these two rows, we located the 10th ommatidium from the edge (Fig. 1b) and then measured three consecutive facets beginning with the third row distant from that point in the ventral direction (small triangles in Fig. 1b). From the same reference point, three consecutive facets were counted beginning with the fifth row distant in the dorsal direction (small circles in Fig. 1b).

For the female adults, there is not a sharp change in facet shape, so an approximate reference point was determined using the medial boundary of the compound eye. The medial portion of the compound eye runs perpendicular to the longitudinal axis of the body and then curves in the ventral half of the eye. The point approximately above this transition was used a reference (black arrow in Fig. 1a). From this point, we counted over three ommatidia and measured the width of two consecutive facets (triangles in Fig. 1c).

For the adult males, we were interested in the number of enlarged dorsal ommatidia that spanned the dorsal region. Starting with the most medial enlarged dorsal facet near the line of demarcation, the number of consecutive ommatidia in a row were counted up the dorsal area until another line of non-hexagonal shaped facets were met (dotted line in Fig. 1d). We used linear regression to characterize the relationship between the various eye features and crossvein length for both males and females.

We also performed a logarithmic transformation on the data for eye size, facet size, and facet number (not shown). By modeling allometry (as reviewed in Shingleton et al. 2007) using the formula $y = ax^b$, (where x is body size, y is the size of the trait of interest, and both a and b are constants), a logarithmic transformation yields a linear equation ($\log(y) = \log(a) + b\log(x)$) where the slope of the line, b, quantitatively describes change in trait size relative to body size. We calculated the value of b to determine the rate of growth for each eye feature relative to body size for eye size, facet size, and facet number using this equation.

Histology. Individuals used for histological work were obtained from a *C. megacephala* laboratory colony established from individuals captured in Miami, FL, in June 2013. Longitudinal cross-sections of compound eyes from a single male and female of approximately equal size were obtained following the methodology described in Meyer-Rochow and Lau (2008). Histological sections were photographed and measurements of the ommatidial lengths were taken using ImageJ software. To get a range of ommatidial lengths, measurements for the female compound eye were taken from the upper, middle, and lower area of the histological sections. Measurements for the male compound eye were taken from upper region of the dorsal area, from near the equator in the dorsal area, from near the equator in the ventral area, and in the lower portion of the ventral area of the histological sections. Body size for these individuals was measured using crossvein length as previously described.

Behavior. Mate detection and capture under low light conditions requires general movement. To determine fly activity during a simulated light/dark cycle incorporating low light conditions, we used an interruption of an infrared beam caused by a moving fly within a confined space (Joshi 1999). Single flies were isolated in a translucent plastic tube lined with foam to limit the range of motion for the fly to the area with the infrared beam. Inside the tube, a moistened cotton ball was placed on one side and sugar was placed on the other (Fig. 3a). The apparatus included a pair of these confinement tubes, each placed between an infrared emitter and receiver. Black poster board visually separated the two tubes, so that flies simultaneously placed individually in each tube could not see each other. A custom python script recorded the time, number, and photocell reading of each beam interruption.

The movement sensors were placed in a DigiTherm Incubator (Tritech Research Inc., Los Angeles, CA) outfitted with a strip of LED lights. The lights were controlled by a microcontroller (Arduino UNO, Arduino, Italy) programmed to simulate a light cycle with natural, gradual transitions. The simulated day cycle consisted of a period of 12 h of darkness, followed by a 2 h increase to a maximum level, a hold at the maximum level for 8 h, followed by a 2 h decrease until the lights went off.

Subjects were from what appeared to be a single egg clutch (based on egg number and arrangement, Wells and Kurahashi 1994). This was done to reduce genetic (Williams and Kokkinn 2005) and eliminate age-related (Gibert et al. 2001, Koh et al. 2006) effects on behavior, as only sibling pairs of equal age were used in the behavior experiments. Individuals within the clutch were reared under the same conditions, except some feeding larvae were removed early to generate smaller individuals. Large and small *C. megacephala* males from the same egg clutch were placed in the incubator to entrain for at least 3 d at the simulated day light cycle. After entrainment, a pair consisting of one large and one small male were simultaneously placed in separate tubes of the movement sensor and observed for two mornings and two evenings. We rotated the different size groups (either "large" or "small" males) between the two sensors to eliminate any bias of one sensor over the other. A total of 10 pairs consisting of a large and small male were used. For a control of the effect of body size independent of the specialized male eye morphology, this work was repeated with 10 pairs of large and small *C. megacephala* females.

Analyses were based on the time that elapsed between 1) the lights turning on and first movement of the individual and 2) the final movement of an individual before the lights turned off. Paired *t*-tests were performed to determine whether the differences in time were significant between body sizes. A two-sample *t*-test, assuming equal variance, was performed comparing the differences in time obtained on both sensors to check for any sensor bias.

The average photocell reading at first crossing after the lights came on for both days was recorded for each fly. Similarly, the average photocell reading at the last crossing in the evening before the lights went off for both days was also recorded for each fly. We converted the photocell readings into light levels by measuring the light level inside the incubator using a light meter (Starlite 2, Gossen, Nürnberg, Germany) at five step increments for the light program. We plotted these values against the average photocell reading across a 5,000 ms integration time (one reading per millisecond) at the same five step increments and used piecewise interpolation for values not directly measured. We used linear regression to characterize the relationship between the average light level and crossvein length.

Determination of Ocellus Size. For the first five pairs from both the male and female behavior experiments, we determined the width of the median and lateral ocelli by taking photos and measuring using Image J. We used linear regression to characterize the relationship between both median ocelli and lateral ocelli width with respect to crossvein length.

Results

Crossvein Length a Suitable Indicator of Body Size. As shown in Fig. 2a, there is a significant positive linear relationship between

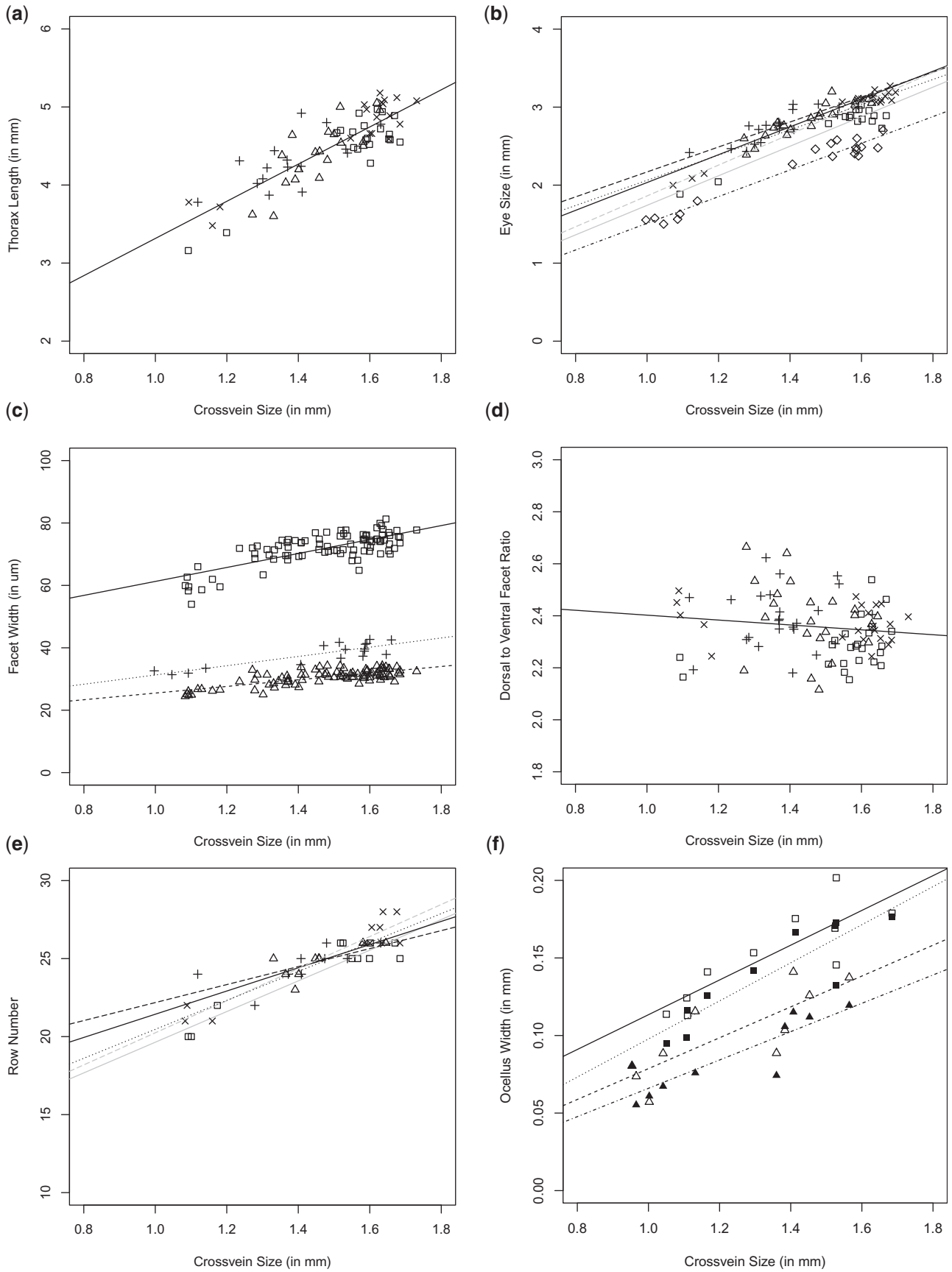

Fig. 2. The relationship between body size and different eye morphological features. (a) The relationship between thorax length and crossvein length. Each group is represented by a different symbol (males: squares for "FIU colony," $n = 21$; triangles for "FIU wild," $n = 19$; plus signs for "Keys wild," $n = 16$; x's for "Marathon Female," $n = 18$). The black regression line is for all groups. (b) The relationship between

crossvein and thorax length (Table 1). From these data, it was determined that crossvein length is a suitable indicator of body size.

Eye Morphology Changes Based on Size. As shown in Fig. 2b, as body size increased, so did the overall size of the eye in males (Table 1) and females (Table 1). Based on the results of the logarithmic regression analysis, this change is nearly isometric ($b = 0.8788$ for all males, $b = 1.1115$ for females). Similarly, individual facet width also increased as body size did for the male dorsal (Table 1), male ventral (Table 1), and female facets (Table 1) (Fig. 2c). This rate of increase, however, was less for each of the male dorsal ($b = 0.4697$), male ventral ($b = 0.5192$), and female ($b = 0.5327$) facets compared with overall eye size. The male dorsal facets were the widest, while the male ventral facets were the narrowest (Fig. 2c). The facet widths from the female eye, lacking a distinct size difference in dorsal and ventral facets, fell in between those of the dorsal and ventral male eye (Fig. 2c). For males, the ratio of the dorsal to ventral facet width was between 2.1 and 2.7 (Fig. 2d), and we found no significant relationship between this ratio and body size (Table 1).

That eye size is increasing at a higher rate than facet size increases implies that the number of ommatidia should also increase as body size does. Our observations support this as Fig. 2e shows that the number of enlarged facets in the dorsal region increased as body size increased (Table 1). The number of ommatidia increases at a rate similar to that of facet size ($b = 0.5393$ for all males). These results showed that eye morphology changes as individuals change in size, with larger individuals having not only larger but more facets.

On the basis of the observed increase in ommatidia, we proposed the following hypothesis: if larger males have larger and an increased number of dorsally enlarged facets, and if these facets are used to increase light capture, then larger males should be better equipped to visually process their environment in conditions of low light than smaller males. Therefore, larger males should show movement when light levels are too low for movement by smaller males. A second behavioral hypothesis was that males should be able to move in lower light levels than females of equal size due to the presence of the specialized region of enlarged dorsal facets.

Male Eye Properties and Population. We used four groups of males: two from different single egg clutches and two wild caught from separate locations. Concerning total eye size, the slope for each individual group line was significantly different from zero (Table 1; Fig. 2b). The two cohorts originating from the same egg clutch had higher correlations than the wild caught individuals (Table 1). None of the groups were significantly different from zero for the dorsal to ventral facet width ratio. Similar to eye size, the slope for each individual group regression line did differ significantly from zero in regards to the number of ommatidia in the dorsal region (Table 1; Fig. 2e). Again a higher correlation existed among the two cohorts from single egg clutches in comparison to the wild population (Table 1).

Table 1. Results of regression analysis for various morphological features (±SE) when compared with crossvein length

Feature	Group	R^2	Intercept	Slope	P
Thorax length	All Males	0.71	0.94(0.27)	2.38(0.18)	<0.001
Eye size	Females	0.95	−0.20(0.13)	1.71(0.09)	<0.001
	All males	0.78	0.45(0.15)	1.62(0.10)	<0.001
	FIU colony	0.89	−0.15(0.28)	1.89(0.18)	<0.001
	FIU wild	0.82	0.25(0.29)	1.78(0.20)	<0.001
	Marathon female	0.97	0.11(0.13)	1.98(0.08)	<0.001
	Keys wild	0.75	0.57(0.32)	1.60(0.23)	<0.001
Facet width	Male dorsal	0.55	38.91(3.22)	22.37(2.17)	<0.001
	Male ventral	0.55	14.84(1.52)	10.65(1.02)	<0.001
	Female	0.75	16.48(3.19)	14.82(2.19)	<0.001
D-V facet ratio	All males	0.019	2.50(0.11)	−0.09(0.071)	0.19
Ommatidia row no.	All males	0.80	11.19(1.09)	9.27(0.74)	<0.001
	FIU colony	0.88	9.81(1.78)	9.83(1.20)	<0.001
	FIU wild	0.61	14.00(3.11)	7.44(2.12)	<0.001
	Marathon female	0.91	9.96(1.64)	10.82(1.09)	0.038
	Keys wild	0.48	16.42(3.20)	5.77(2.26)	0.008
Median ocellus width	Males	0.71	0.0013 (0.034)	0.11(0.025)	0.002
	Females	0.66	−0.021(0.031)	0.10(0.025)	0.004
Lateral ocellus width	Males	0.79	−0.025(0.030)	0.12(0.022)	<0.001
	Females	0.76	−0.025(0.022)	0.09(0.018)	<0.001

body size and eye size. Each group is represented by a different symbol (males: squares for "FIU colony," $n = 16$; triangles for "FIU wild," $n = 20$; plus signs for "Keys wild," $n = 18$; x's for "Marathon Female," $n = 19$; females: diamonds, $n = 19$). The dotted black regression line is for the entire group of males. The gray regression lines are for the two groups originating from a single egg clutch. The black regression lines (with the exception of the dotted one) are for the wild caught groups. The solid line indicates an origin on the FIU campus. A long dashed line indicates an origin from the Florida Keys. The dot-dash line is for females. (c) The relationship between body size and facet width. The squares are male dorsal facets ($n = 91$). The triangles are male ventral facets ($n = 91$). The plus signs are female facets ($n = 17$). (d) Dorsal to ventral ratio versus crossvein size for male individuals. Each group is represented by a different symbol (males: squares for "FIU colony," $n = 23$; triangles for "FIU wild," $n = 22$; plus signs for "Keys wild," $n = 24$; x's for "Marathon Female," n = 22). (e) The relationship between body size and height of the dorsal area in ommatidial rows. Each group is represented by a different symbol (males: squares for "FIU colony," $n = 11$; triangles for "FIU wild," $n = 10$; plus signs for "Keys wild," $n = 9$; *x*'s for "Marathon Female," $n = 11$). The dotted regression line is for the entire group of males. The gray regression lines are for the two groups originating from a single egg clutch. The black regression lines (with the exception of the dotted one) are for the wild caught groups. The solid line indicates an origin on the FIU campus. A dashed line indicates an origin from the Florida Keys. (f) The relationship between body size and ocellus width. The squares are for males, and the triangles are for females. Empty shapes correspond to median ocellus, filled in shapes correspond to lateral ocellus. The solid regression line is for the median male ocellus. The dotted line is for lateral male ocellus. The dashed regression line is for the median female ocellus. The dot-dash regression line is for the lateral female ocellus.

Ommatidium Length. As listed in Table 2, the ranges of ommatidial lengths were similar in the male dorsal region and the female. The male ventral region had the largest maximum ommatidial length.

Behavior. To test the hypothesis of large males moving earlier in the day than small males, we measured activity at different light levels through a simulated day. As shown in Fig. 3b, larger males moved significantly earlier in the morning than smaller males did ($t = 4.1778$, df = 9, $P = 0.002384$). On only two of the 20 mornings was the smaller male the first to move. In the evening, however, there was not a significant difference in movement times based on body size ($t = -0.0654$, df = 9, $P = 0.9493$). A similar pattern was seen in the females, with larger individuals moving earlier in the morning ($t = 3.8333$, df = 9, $P = 0.004008$, Fig. 3b). For the females, on only four occasions out of the 20 mornings did the smaller female move before the larger one. For female movement in the morning, there was an outlier more than 2.5 times the upper quartile. When removed, the difference in movement was still significant ($t = 5.5835$, df = 8, $P < 0.001$) in the morning. There was not a significant difference in time during the evening ($t = -0.2290$, df = 9, $P = 0.8240$). There was no significant difference in times between the two sensors ($t = -0.6920$, df = 38, $P = 0.4931$), showing no effect of the sensor.

The relationship between the average light level at first movement and body size revealed an inverse relationship in both males (Table 3) and females (Table 3, Fig. 3c). For both sexes, larger individuals moved

Table 2. Ommatidial length ranges (minimum to maximum) for the compound eyes in a female and a male *C. megacephala* of similar size

Sex	Eye region	Ommatidial length range (μm)	Crossvein length (mm)
Female	—	238.29–257.26	1.50
Male	Dorsal	242.90–252.96	1.48
Male	Ventral	237.94–265.98	1.48

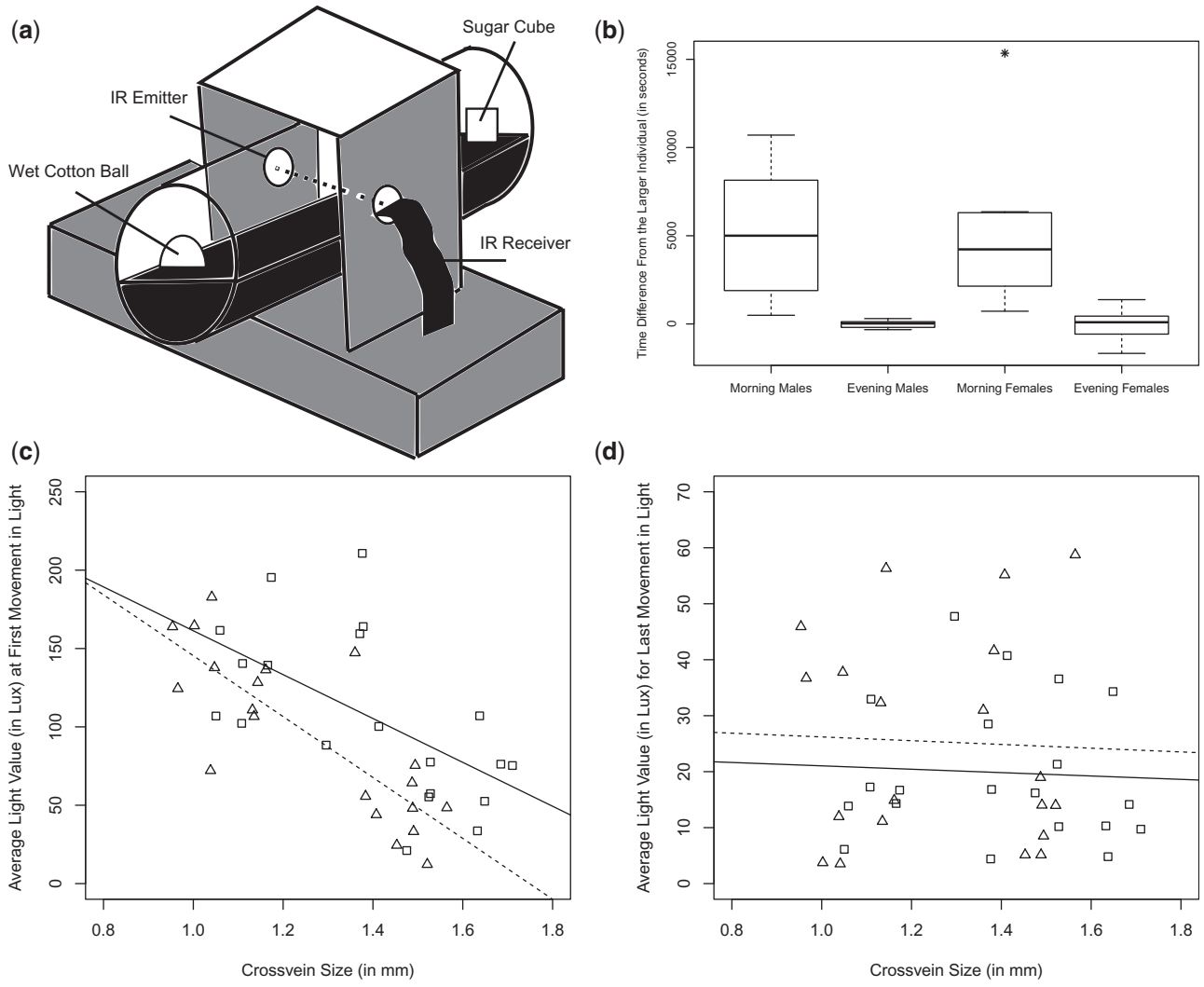

Fig. 3. Behavioral analysis of *C. megacephala* daily movement. (a) Drawing of one infrared sensor used. For the final experimental design, two of these were present on the same base to allow for simultaneous measurements from two different flies. (b) Comparison of the time differences between first (in the morning) or last (in the evening) movement between smaller and larger individuals ($n = 10$ pairings, two replicates per pairing). The thick bar represents the median. (c) The average light value at the time of the initial cross in the morning after the lights came on. The squares represent the males ($n = 20$) and triangles represent the females ($n = 20$). The solid line is the regression for males. The dashed line is the regression for females. (d) The average light value at the time of the last cross in the evening before the lights turned off. The squares represent the males ($n = 20$) and triangles represent the females ($n = 20$). The solid line is the regression for males. The dashed line is the regression for females.

Table 3. Results of regression analysis for light level at first movement after lights came on or last movement before lights turned off (±SE) compared with crossvein length

Time light level taken	Group	R^2	Intercept	Slope	*P*
First movement in light	Female	0.65	339.9(42.43)	−194.6(33.11)	<0.001
	Male	0.34	301.28(65.01)	−139.96(46.10)	0.007
Last movement in light	Female	0.001	29.55(26.30)	−3.35(20.53)	0.872
	Male	0.003	24.10(18.95)	−3.02(13.44)	0.825

at lower light levels. Comparison of males and females of similar size showed that females moved at lower average light levels (Fig. 3c). For the evening, however, we did not find a significant relationship between body size and light level at last movement for both males (Table 3) and females (Table 3, Fig. 3d).

Ocellus Size. As can be seen in Fig. 2f, the width of the median ocellus increased as body size increased for both males and females (Table 1). Similarly, the width of the lateral ocellus also increased. For both types, males have wider ocelli than females.

Discussion

***C. megacephala* Males of Different Sizes have Morphologically Different Eyes.** By taking into account body size, a factor absent in previous work on *C. megacephala* male eye morphology (van Hateren et al. 1989, Stavenga et al. 1990, Sukontason et al. 2008), we demonstrated that not only do eye size and ommatidium widths increase as the individual gets larger, but the number of dorsally enlarged ommatidia also increases. For an insect, enlarged facets lead to the possible undersampling of an image, since large facets use up the finite surface area of the compound eye. van Hateren et al. (1989) described two possible solutions for undersampling in *C. megacephala*: increasing the number of ommatidia or increasing the rhabdomere diameter. They found *C. megacephala* increased the rhabdomere diameter (van Hateren et al. 1989). The observations made in our current work show that larger males may also address the issue of undersampling with a larger number of ommatidia in the dorsal area.

We did not find a significant difference in dorsal to ventral facet width as body size increases. The range in dorsal to ventral facet width we observed (approximately 2.1–2.7) is rather less than the previous report of the dorsal facets being four times larger than the ventral facets (van Hateren et al. 1989). A reason for this may be that we measured facet widths from a different area than van Hateren et al. (1989), who did not specify exact facet location, and that facet size changes based on location. In the blow fly *Calliphora vicina* (=*Calliphora erythrocephala)*, e.g., there is a change in facet size across different regions related to spatial acuity (Land and Eckhart 1985).

The relative rates of growth observed in male *C. megacephala* between body size and eye size, facet size, and ommatidia number are similar to the values reported across 15 different species of bees (Jander and Jander 2002) and within individuals of varying size for the same bee species (Spaethe and Chittka 2003). The similarity in results is particularly interesting considering that the previously described bee species have compound eyes containing facets with gradual changes in size, while male *C. megacephala* has a specialized region of enlarged facets sharply differentiated from those on the rest of the eye. Whether these properties would be similar in compound eyes with and without regionalization was discussed in Jander and Jander (2002), and here we provide one example where they are. Since *C. megacephala* does not experience a change in resolution between the enlarged dorsal facets and the smaller ventral facets, however, comparisons between our results and a compound eye with regionalization that is associated with a change in resolution should be considered.

Higher Correlation Within a Population. We found a higher correlation between body size and morphological features for the two groups originating from a single egg clutch as opposed to the two wild caught populations. This was expected as the two single egg clutch groups had similar genetic backgrounds and were reared under known environmental conditions. For the wild caught populations, however, both the genetic background and environmental conditions during development were unknown. This may explain why the female correlation values were higher than the males, since the females used in this work all came from the same egg clutch, while the males consisted of both individuals from the same egg clutch and wild caught ones. These findings support the idea that studies measuring the relationship between a certain trait and body size in insects should use genetically similar individuals to observe the entire range of trait morphologies one genotype can produce when faced with different developmental environments (discussed in Emlen and Nijhout 2000).

Another factor that likely influenced the higher correlation for the groups originating from a single egg clutch is that there was less overall size variation. While in the single egg groups we were able to generate a greater size range including individuals smaller than we found in our wild caught populations, these single egg groups consisted of either very small or very large individuals. This was in contrast with the wild groups that had an overall narrower size range but more individuals that would be considered intermediate in size. Future work should consider trying to generate a gradient of sizes for the single egg groups to see if the higher correlation is still evident in comparison to a wild population.

Total Body Size, Not Specialized Male Eye Morphology, Is Important for Being Active in Low Light Conditions. Our hypothesis that larger males would be able to move at lower light levels than smaller males was supported in the morning, but not in the evening. However, this same pattern of behavior was exhibited in females, who do not have the specialized dorsal region of facets. In fact, females actually moved at lower light levels than males of similar size despite having smaller overall eye sizes. For this reason, we cannot attribute the early movement in larger males to their specialized eye morphology. The difference in movement appears to be attributed to being a larger individual and may be the result of larger individuals simply having larger eyes that capture more light (Jander and Jander 2002, Kelber et al. 2006).

Previous work in the blow fly *Ca. vicina (=Ca. erythrocephala)* found it took longer for activity to increase as light levels increased than it did for activity to be reduced as light levels decreased (Digby 1958). This is consistent with our current work as we observed crosses at lower light levels as the lights were decreasing in comparison to when they were increasing.

Blow flies are generally considered to be diurnal (Anderson 2001). A blow fly relies on carrion as a place to oviposit, feed, and find mates (Norris 1965, Erzinçlioglu 1996), so movement earlier in the day may confer an individual advantage because arriving first on carrion that has died during the night might help a blow fly avoid significant competition that would arise later in the day. Furthermore, by arriving early, a blow fly would have earlier access to potential mates. In the evening, however, blow flies of all sizes have had all day to visit carrion and search for mates, so staying out later is not as crucial. This suggests that being a larger blow fly is advantageous as it allows for early morning movement.

Internal Compound Eye Factors Affecting Sensitivity. Previously, van Hateren et al. (1989) found the dramatic size change in the external facet widths of the dorsal male eye compared with the facet widths in

the ventral portion also occurred in the photoreceptors. Specifically, rhabdomere widths in the dorsal region could be in upwards of two and a half times larger than those found in the ventral region (van Hateren et al. 1989). Despite this change, we did not observe a difference in sensitivity as measured by movement at lower light levels between males and females. While diameter is considered one of the most important factors affecting sensitivity, the length of the receptor can also play a role (Land and Nilsson 2012). One possible explanation for why the sensitivities are similar is that the male dorsal area, while containing larger diameters, has a shorter ommatidial length. We found, however, that ommatidial lengths were approximately equal in the female compound eye and the dorsal region of male compound eye, and actually longer in the male ventral region.

While in the male ventral portion of the eye the rhabdomeres extend nearly to the basal membrane, the rhabdomeres in the dorsal region taper well before reaching the basal membrane (van Hateren et al. 1989). This in turn makes it difficult to obtain the rhabdomere length in this region. As such, we were unable to measure rhabdomere length in this study. Future work should investigate rhabdomere length in the male dorsal region as a wider, but shorter rhabdomere length may be similar in sensitivity to a narrower but longer rhabdomere.

Other External Factors Affecting Sensitivity. Previous work has indicated that the ocelli can play a role in the onset of activity in response to light changes (Wunderer and De Kramer 1989). That males have larger median and dorsal ocelli than their female counterparts, however, provides support that the lack of difference in onset of activity cannot be attributed to the ocelli.

Future Behavioral Research. Because we confined flies to small spaces, the movement we measured here was walking. Future studies should investigate designs that allow for flight, as we hypothesize that there may be different light thresholds for walking movement versus flying movement. Walking flies have the advantage of having tactile stimulation to help their movement and location. In flight, however, flies must largely rely on visual information (Theobald et al. 2007). There may well be two separate light level thresholds, one for walking and the other for flight.

Furthermore, we used an artificial light cycle created by LEDs. While this allowed for complete control over the light cycle values, it may not accurately reflect natural lighting conditions. The LEDs used were poorer in long wavelength light than shorter wavelength light. van Hateren et al. (1989) described the wider dorsal rhabdomeres were capable of higher modes of longer wavelength, so possibly the ability of the dorsal facets to receive light was reduced. Now that the pattern of early movement for larger individuals has been established in a laboratory setting, future work should utilize an experimental design outdoors to incorporate more natural lighting conditions.

Narrowing Down the Functionality of the "Bright Zone." Previously, van Hateren et al. (1989) hypothesized that the "bright zone" was used to either track females in low light levels or to search for them at further distances in higher light levels. The observations from this work seem to support the latter, as *C. megacephala* males were not more active in lower light levels than females of similar size. These findings suggest that male eye morphology is not used in moving at lower levels of light, as would be required for low light level mate tracking. Although it remains possible that the specialized male eye morphology could be used to track females in low light conditions, without moving earlier to sites with active females they are unlikely to take advantage of it. Future work should investigate the possibility that this eye morphology is used to track females at higher levels of luminance at further distances, as also hypothesized by van Hateren et al. (1989).

Overall Conclusions. There are a number of reasons a blow fly would benefit from a larger adult size. For example, larger males are capable of mating with a wider size variety of females (Stoffolano et al. 2000) and can make the females they mate with less receptive to other mates in comparison to smaller males (Cook 1992). Similarly, larger females have been shown to be able to produce more eggs (Wall 1993). Here, we have shown another reason why it is beneficial to be a larger blow fly based on visual properties, as larger individuals can move earlier in the morning than their smaller counter parts. Additionally, we have described a new aspect of the behavioral ecology of *C. megacephala*. *C. megacephala* is a fly of forensic importance, and it is acknowledged that little is known about the behavior of forensically important blow flies, especially away from a corpse (Tomberlin et al. 2011).

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