

Spatial vision in band-winged grasshoppers (Orthoptera: Acrididae: Oedipodinae)

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ABSTRACT

Visual acuity, the ability to resolve fine spatial details, can vary dramatically between and within insect species. Body-size, sex, behavior, and ecological niche are all factors that may influence an insect's acuity. Band-winged grasshoppers (Oedipodinae) are a subfamily of grasshoppers characterized by their colorfully patterned hindwings. Although researchers have anecdotally suggested that this color pattern may attract mates, few studies have examined the visual acuity of these animals, and none have examined its implications on intraspecific signaling. Here, we compare the visual acuity of three bandwing species: *Dissosteira carolina*, *Arphia pseudonietana*, and *Spharagemon equale*. To measure acuity in these species we used a modified radius of curvature estimation (RCE) technique. Visual acuity was significantly coarser 1) in males compared to females, 2) parallel to the horizon compared to the perpendicular, and 3) in *S. equale* compared to other bandwings. Unlike many insect families, body size within a species did not correlate with visual acuity. To examine the functional implications of these results, we modeled the appearance of different bandwing patterns to conspecifics. These results suggest that hind-wing patterning could only be used as a signal to conspecifics at short distances (<50cm). This study furthers the exploration of behavior and the evolution of visual systems in bandwings.

Key words: *Oedipodinae*, *visual acuity*, *band-winged grasshopper*, *compound eyes*, *protean defense*, *visual ecology*, *ethology*

INTRODUCTION

An animal's behavior is limited by the information its sensory systems can gather (Partan and Marler 2002; Jordan and Ryan 2015). Therefore, understanding what information an animal lineage perceives is critical to understanding how it can adapt to an environment (Jordan and Ryan 2015). Notably, the sensory abilities of a non-human animal can drastically differ from our own, and some species cannot accomplish tasks that a human could (Jakob von Uexkùe 2001). Thus, we must account for an animal's sensory abilities when assessing a behavior's adaptive quality (Romer 1993; Jordan and Ryan 2015). Selection on sensory abilities in the environment can also lead to preferences for sexual signals related to those abilities (Boughman 2002; Maan et. al 2006; Ryan and Rand 1990). This bias for specific signal form, known as sensory drive, can then lead to speciation events within a population (Ryan and Cummings 2013; Endler and Basolo 1998). Thus, a complete understanding of evolution in an animal lineage requires an understanding of their signaling and sensory abilities (Maan et. al 2006; Cummings 2007).

Like other sensory systems, animal eyes differ greatly in their capacity to gather information (Brandley pers. com.; Bennett and Théry 2007; Briscoe and Chittka 2001). In invertebrates, including insects, the mechanism of vision is the compound eye (Land 1997). This eye is composed of small optical units known as either ommatidia (*i.e.* facets) each with their own lens and photo-sensing capabilities (Barlow 1952; Kirschfeld 1976). Compared to human eyes, the design of the compound eye limits spatial resolution (Kirschfeld 1976; Land 1997; 1999a), affecting tasks such as predator identification

(Belovsky et al. 1990), orientation (Land 1997), mate choice, and mate recognition (Burton and Laughlin 2003). One measurement of spatial vision is visual acuity (VA). VA is the minimum angle in which an animal can fully separate a pair of black and white stripes, and in the compound eye VA is determined by the angular relationship between facets (Barlow 1952; Kirschfeld 1976). A compound eye can improve acuity by two mechanisms: 1) making the eye surface flatter, or 2) making facet diameter smaller (Barlow 1952; Kirschfeld 1976). Both processes decrease the angle between adjacent facets, making VA finer (Barlow 1952; Kirschfeld 1976). Although an insect can improve acuity through facet size decreases, these decreases are limited by diffraction and reduce light capture (Barlow 1952; Warrant 2004). Therefore, researcher need to be aware of the constraints on insect VA when exploring their visually derived behaviors.

Multiple trends help explain the variation in insect VA (ranging from: 0.48° to 67.3° ; Land 1997; Brandley pers. com.). Similar to other animals (Kiltie 2000; Veilleux 2014), increases in insect body size lead to finer VA both within and between families (Land 1997; Jander and Jander 2002). Changes in acuity also correlate with various ecological factors. For instance, nocturnal insects typically have coarser VA to improve light capture in dimly lit environments (Jander and Jander 2002; Horridge 1978; Land et al. 1999; Warrant and Dacke 2011). Insects with behaviors that require fine spatial resolution, like complex flight patterns or in air predatory behavior, have finer acuity as well (Land 1997). For example, dragonflies have fine VA, which along with high sampling rate, allow these insects to visually track and capture prey in mid-air (Land 1997; Olberg 2000; 2007). Compound eyes can also be subject to regional variation in acuity across a single eye, giving an insect

finer acuity in eye regions that are behaviorally relevant (Land 1989; Perl and Niven 2016; Rutowski and Warrant 2002). This variation can improve mate detection (Burton and Laughlin 2003), flight abilities, and many other behaviors (Land 1997).

Among grasshoppers, most visual work has been performed in one species, the locust (*Locusta migratoria*). Locusts have apposition compound eyes (Land 1997), which have a zone of fine VA in the eye center (Krapp and Gabbiani 2005; Rutowski and Warrant 2002). An additional area of their eye aids in predator avoidance by being sensitive to looming objects (Santer et al. 2012). This area has limited crossover with fine VA zone (Krapp and Gabbiani 2005). From a small group of individuals, locust VA has been calculated to be $\sim 1.8^\circ$ (Horridge 1978), but this measurement does not account for regional variation in acuity. Similarly, short-horned grasshoppers (family *Acrididae*), like the locust, generally have an acute zone at the eye's center, associated with their flying behavior (Horridge 1978; Land 1999a).

Locusts are a species of band-winged grasshopper (Oedipodinae), a subfamily of *Acrididae* containing about 200 species (Otte 1970; Willey and Willey 1969). These grasshoppers are characterized by their colorful hind-wings, but it is still unclear why their hind-wing patterning and coloration evolved. Researchers have posited that: 1) they may be a part of a mating display and/or 2) act as a predator deterrent (Otte 1970). Bandwings likely use multimodal mating displays, which may include chemical, tactile, and visual signals (Willey and Willey 1969; Otte 1970; Candolin 2003). Behaviors associated with mating interactions at a distance typically include flight patterns and clicking sounds known as a

flight crepitation, but at short distances consist of physical contact and possibly pheromones (Willey and Willey 1969). Crepitation at a distance may function as a visual signal in courtship displays (Otte 1970). Although behavioral evidence is lacking, Oedipodinae conspecific detection distances have been suggested to range from around 30cm (Willey and Willey 1969) to 3m (Niedzlek-Feaver 1995). Visual recognition may occur at even shorter distances as males typically spend time searching for their mate on the ground (Niedzlek-Feaver 1995). While other senses may be used in mate detection, this data suggests visual recognition may only occur at short range of distances. However, no study of visual mechanisms has been undertaken in bandwings, besides *L. migratoria*, and no work has explored the importance of hind-wing patterning and other visual signals in their courtship displays.

This hind-wing patterning and coloration may also function as a protean predator defense in grasshoppers (Cooper 2006; Cott 1940). Protean behavior is an erratic action, which often involves bright flashes of color that confuses a predator and makes it difficult to predict prey movement (Humphries and Driver 1970). Common grasshopper predators include birds like the Western Meadowlark (*Sturnella neglecta*), the Grasshopper Sparrow (*Ammodrammus savannarum*), and Kingbirds (*Tyrannus tyrannus* and *T. verticalis*) (Belovsky et al. 1990). Rodents like *Peromyscus maniculatus* and *Microtus pennsylvanicus*, spiders in the families *Clubionidae* and *Lucosidae*, and ants are also known to prey on grasshoppers (Belovsky et al. 1990). Grasshopper flight behavior increases incidence of predation, and thus Oedipodinae experience more predator interactions than other groups of species (Butler 2013). Bandwings initiate escape behavior

at greater distances than other grasshoppers as well (Butler 2013), perhaps as an adaptive response to this trend. This behavior suggests that Oedipodinae do not rely on their cryptic coloration while moving (Butler 2013), and instead use their hind-wing patterning as a flight based protean defense mechanism (Cooper 2006; Humphries and Driver 1970; Cott 1940; Bateman and Fleming 2014).

The following study explores the mechanisms of Oedipodinae VA and the behavioral implications of spatial vision on bandwing behavior. Here we investigate VA in bandwings, including how VA varies between different species and sexes, how it varies in visual axes perpendicular and parallel to the horizon, and how bandwing VA compares to non-bandwing species. Finally, we use these VA results to model how bandwings perceive the hind-wing patterning of conspecifics and make behavioral inferences from our findings regarding the function their hind-wing patterning.

METHODS

Study Organisms

As male bandwings are more active than females (Willey and Willey 1969), more males than females were sampled in this study. *Dissosteira carolina* (n = 16 males and 6 females) specimens were collected on the lawns of the Colorado College campus (38°50'53"N 104°49'26"W) in July of 2016. *Arphia pseudonietana* (n = 18 males and 7 females), *Spharagemon equale* (n = 15 males and 11 females), and all other specimens were collected at a high-altitude grassland site (38°50'34"N 104°28'30"W) in October of 2016. To

compare Oedipodinae to other subfamilies and species, *Melanoplus gladstoni* (n = 2 males and 2 females), *Melanoplus bivittatus* (n = 4 females), *Aeoloplides turnbulli* (n = 6 females) *Schistocerca alutacea* (n = 1 female) and *Brachystola magna* (n = 1 female) were also analyzed. Grasshoppers were killed using ethyl acetate and then stored at approximately -18°C prior to imaging. Mass (g), length (mm), and sex were recorded for each individual.

Imaging

Imaging methods were adapted from Bergman and Rutowski (2016). Eye images were produced using a microscope (M28Z Zoom Stereo Binocular Microscope; Swift; Carlsbad, CA) paired with a digital camera (14MP USB3.0 Real-Time Live Video Microscope USB Digital Camera; AmScope; Irvine, CA). Images were recorded with AmScopeX for Mac MU (MW Series 05/26/2016; AmScope; Irvine, CA) under diffuse lighting conditions (LED312DS; Fotodiox; Gurnee, IL). For ease of image capture, specimens were first decapitated and heads were placed on the microscope stage. The most intact eye on each specimen was used for imaging. All images were taken at a 4X magnification, excluding the larger eyes of both *B. magna* (3X) and *S. alutacea* (2X). Three images of each eye were collected for analysis: one lateral view to explore acuity in the visual axis perpendicular to the horizon (*Figure 1A*), one dorsal view to explore acuity parallel to the horizon (*Figure 1B*), and one anterior view (*Figure 1C*) to estimate facet density. For consistent positioning, physical attributes were used for orientation; the inside eye edge was used for lateral images, the top of the eye was centered for dorsal images, and the center of the eye surface was used for anterior images. To capture eye shape, images were focused on the outside edge of the eye in lateral and dorsal images.

Image Analysis

Image analysis techniques were adapted from the Radius of Curvature Estimation method (RCE; Bergman and Rutowski 2016). All images were analyzed using Image J (1.50i; National Institutes of Health; Bethesda, MD).

VA was derived from lateral images to determine VA parallel to the horizon (parallel VA) and from dorsal images to determine VA perpendicular to the horizon (perpendicular VA). Measurements were performed at the eye center along both the lateral and dorsal eye edge. First, to calculate interommatidial angle ($\Delta\Phi$), the angle (α) of two lines drawn perpendicular to the radius of curvature on the eye edge was derived (*Figure 1A;B*). Distance was then calculated between two points created by the intersection of lines perpendicular to the eye edge (b). Average facet density (D) was then measured on the flattest eye surface in anterior images (*Figure 1C*). As facets were found to be the same size across the eye surface of each individual grasshopper (Horridge 1978), average facet diameter was calculated from two perpendicular rows of ten facets at the eye center. The number of facets within the measured area was calculated by dividing D by b . The $\Delta\Phi$ was then calculated by dividing α by the number of facets. Finally, to determine VA in degrees, $\Delta\Phi$ was doubled.

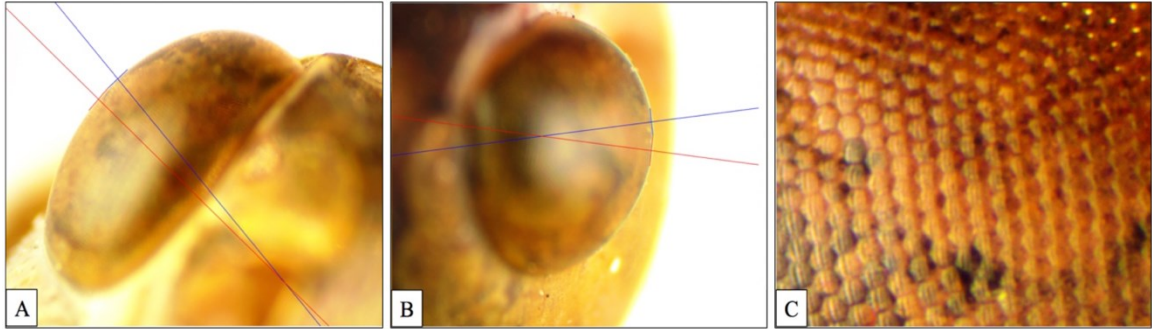


Figure 1: An image set used in data analysis. Images are of a *D. carolina* eye. A) A lateral view of the eye, with the eye edge in focus. Lines have been drawn at the center of the eye perpendicular to the radius of curvature. B) A dorsal view of the eye, with the eye edge in focus. Lines have been drawn at the center of the eye perpendicular to the radius of curvature. C) An anterior view of the eye, with facets in focus at the eye center. Average facet diameter was determined from this image.

Analysis of Regional Variation in Visual Acuity

As a form of exploratory data analysis into regional variation VA, the above analysis methods were performed across the entire eye edge, both perpendicular and parallel to the horizon (n = 1 female and 1 male per species). First, the center of the base of the eye was identified and a line 90° from the base was then drawn to the eye edge; this point was deemed 0°. Next, acuity was measured at 10° intervals across the eye surface from the 0° line. Acuity was measured in this way until the image of the eye edge was no longer in focus (*Figure 2*).

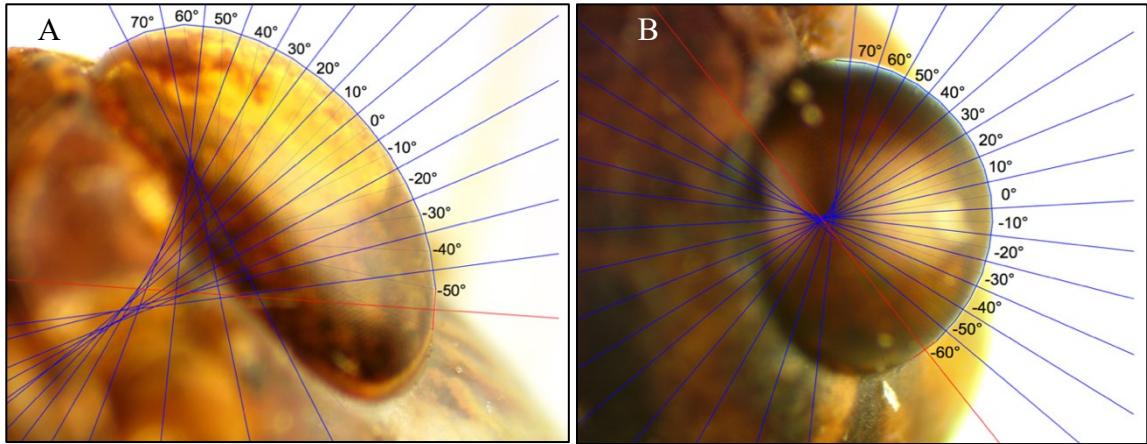


Figure 2: An image set used in regional acuity analysis in *D. carolina*. Measurements were taken at 10° intervals around the eye edge. A) Perpendicular VA was measured from a lateral eye image and B) Parallel VA was measured from a dorsal eye image.

Data Analysis

As it is less affected by the immediate condition of the animal, length was a more reliable measurement of body size than mass. Linear regressions were performed (Microsoft Excel for Mac 2017; 15.32; Microsoft; Santa Rosa, CA) to elucidate trends between acuity, body length and facet size. Separate regressions were performed for males and females within a species, as well as for perpendicular and parallel VA.

To evaluate differences between bandwings in perpendicular and parallel VA between each species, within each species, and between sexes within each species, unpaired two-tail t-tests were performed with a 95% confidence interval ($\alpha=0.05$).

Finally, to explore what factors statistically influence acuity within each axis, a generalized linear model (GLM) using the R statistical programming language was utilized to examine potential differences in species, sex, facet diameter, mass, and body length. GLMs were

not performed on non-bandwing grasshoppers due to small sample size. GLM predicted values were produced for both males and females within each bandwing species. To remain conservative in analysis, model selection was determined by the most parsimonious relationships using the Bayesian information criterion (BIC; R Core Team 2013).

Visual Models

To explore visual perception of conspecific hind-wing patterning, images of hindwings were modeled with *S. equale* and *A. pseudonietana* visual acuity at behaviorally relevant distances following the methods of Johnsen and Caves (in prep.). *D. carolina* was excluded from analysis due to issues with hind-wing images. Model images were created by integrating GLM predicted values of perpendicular and parallel VA for both males and females within each species (Figure 3). To determine how image quality changes with distance, images were modeled at 10, 25, 50, and 100cm. Unmodified images were also used for comparative purposes.

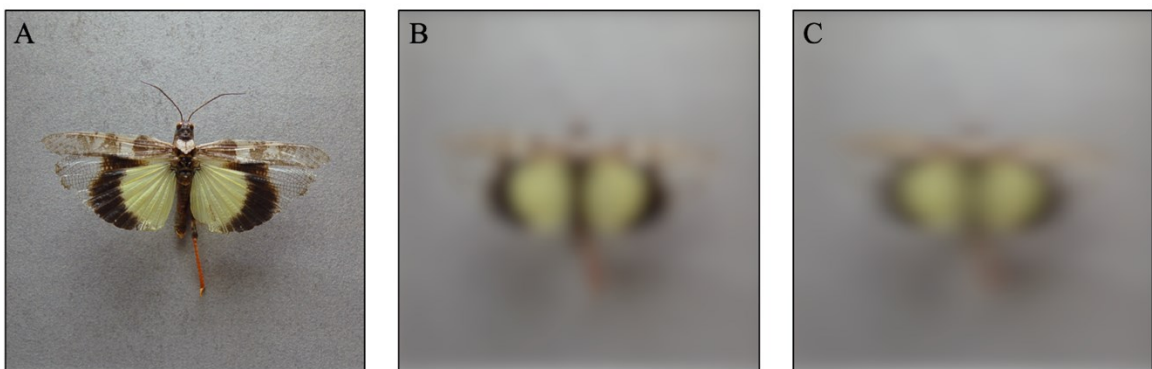


Figure 3: The influence of differences in perpendicular and parallel VA on image quality. A) An unmodified image of *S. equale*. B) *S. equale* when viewed with perpendicular VA of a female *S. equale* at 10cm. C) Same image as B, but also accounting for coarser parallel VA.

RESULTS

Visual Acuity

D. carolina, *A. pseudonietana*, and *S. equale* showed similar VA values, with females having finer acuity than males overall ($p < 0.05$; see GLM section). In general non-bandwings had finer VA than bandwings (Figure 4), but due to small sample size the statistical significance of these results cannot be commented on.

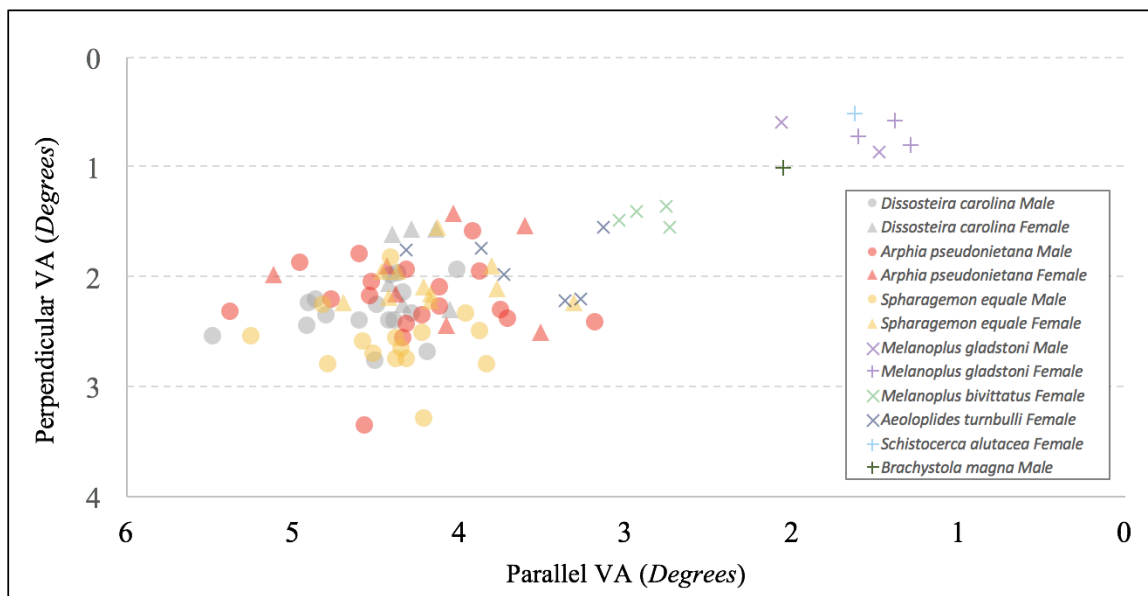


Figure 4: The perpendicular and parallel VA of every individual sampled. Bandwing males (●) and females (▲) are grouped on the lower left corner. Non-bandwing males (+) and females (X) are finer than bandwings and trend towards the upper right corner.

Comparison of Visual Acuity Perpendicular and Parallel to the Horizon

For each species for which statistical analysis was appropriate, average perpendicular VA was significantly finer than parallel VA (*Table 1*).

Table 1: Intraspecific Difference in Average VA Perpendicular and Parallel to the Horizon.

Species	Perpendicular VA	Parallel VA	N	df	T	p
<i>D. carolina</i>	2.21°	4.47°	22	21	2.08	$p < < 0.05$
<i>A. pseudonietana</i>	2.17°	4.25°	25	24	2.06	$p < < 0.05$
<i>S. equale</i>	2.37°	4.28°	26	25	2.06	$p < < 0.05$
<i>M. gladstoni</i>	0.71°	1.56°	5	4	2.78	$p < 0.05$
<i>M. bivittatus</i>	1.45°	2.86°	4	3	3.18	$p < 0.05$
<i>A. turnbulli</i>	1.91°	3.60°	6	5	2.57	$p < 0.05$
<i>S. alutacea</i>	0.51°	1.62°	1	-	-	-
<i>B. magna</i>	1.07°	2.05°	1	-	-	-

Generalized Linear Model

GLM predicted values can be found in *Table 2*.

Table 2: GLM predicted VA values for band-winged grasshoppers.

Species	Sex	Predicted Per. VA	Predicted Par. VA
<i>D. carolina</i>	male	2.33°	4.53°
	female	1.91°	4.32°
<i>A. pseudonietana</i>	male	2.29°	4.31°
	female	1.87°	4.09°
<i>S. equale</i>	male	2.55°	4.37°
	female	2.13°	4.16°

When testing perpendicular VA, the most parsimonious model included species and sex as factors (*Table 3*). Within this model, *S. equale* had significantly coarser perpendicular VA than other bandwing species ($p < 0.01$) and males had significantly coarser perpendicular VA than females ($p < 0.01$).

When testing parallel VA, the most parsimonious model included both sex and facet diameter as factors. Within this model males had significantly coarser vision than females, and larger facet diameter led to coarser VA (*Table 3*; $p < 0.01$). To further explore the importance of facet diameter in this model, male bandwing parallel VA and facet diameter were compared using linear regression, and a weak but significant correlation was observed (*Figure 5*; $p < 0.05$; $R^2 = 0.11$). The effect of male facet size on model-predicted values of parallel VA followed a similar trend (*Table 4*).

Table 3: GLMs for the effect of morphological factors on VA.

<i>Model</i>	<i>df</i>	<i>BIC</i>	<i>Log-lik</i>	<i>P</i>
<u>Perpendicular to the Horizon</u>				
<i>Per. ~ Species</i>	70	77.93	-30.38	-
<i>Per. ~ Species + Sex</i>	69	57.98	-18.26	<0.01
<i>Per. ~ Sex</i>	71	58.93	-23.03	<0.01
<i>Per. ~ Species x Sex</i>	67	63.78	-16.87	.27
<i>Per. ~ Species + Sex + Length</i>	68	59.76	-17.01	.12
<i>Per. ~ Species + Sex + Facet</i>	68	62.01	-18.13	.62
<i>Per. ~ Species + Sex + Mass</i>	68	60.39	-17.32	.18
<u>Parallel to the Horizon</u>				
<i>Par. ~ Species</i>	70	95.08	-38.96	.07
<i>Par. ~ Sex</i>	71	90.38	-38.76	-
<i>Par. ~ Species + Sex</i>	69	95.11	-36.83	.15
<i>Par. ~ Sex + Length</i>	70	92.16	-37.50	.12
<i>Par. ~ Sex + Facet</i>	70	85.07	-33.95	<0.01

(The most parsimonious GLM for each category is in bold. Each model is compared with the most parsimonious model located above within each category. See methods for description of variables.)

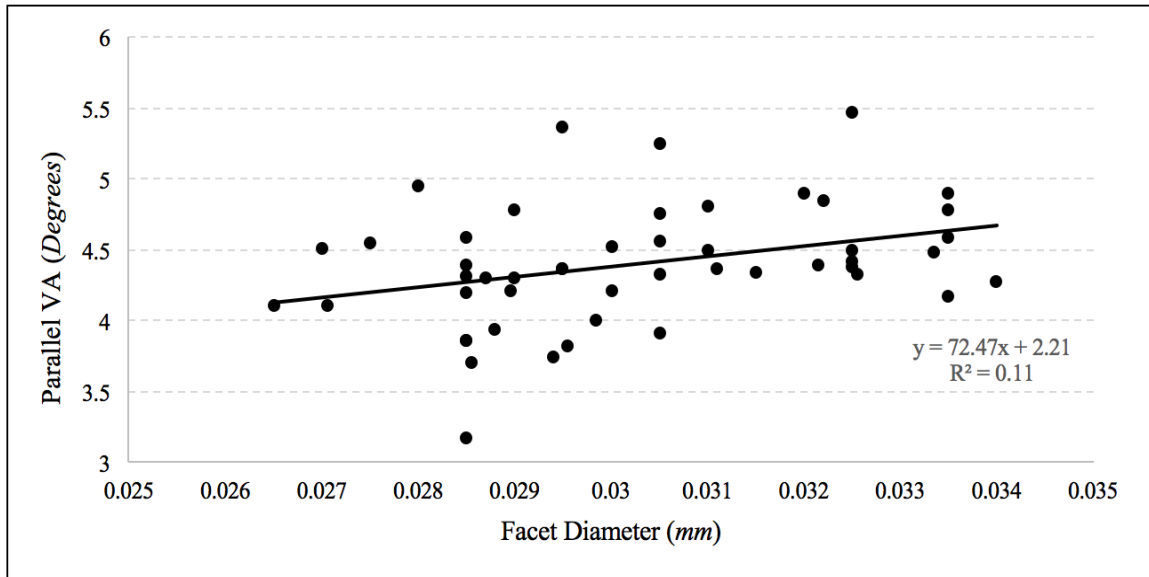


Figure 5: In males, larger facet diameters led to coarser parallel VA (linear regression; $p < 0.05$; $R^2 = 0.11$).

Table 4: Parallel VA GLM, including sex and facet size as factors, predicted parallel VA values based on facet diameter for male bandwings.

Facet Diameter (mm)	GLM Predicted Parallel VA
0.026	4.15°
0.027	4.21°
0.028	4.28°
0.029	4.35°
0.030	4.41°
0.031	4.48°
0.032	4.55°
0.033	4.61°
0.034	4.65°

Grasshopper Length and Visual Acuity

Average species body length did not correlate with either perpendicular VA (*Figure 6A*; $R^2= 0.19$; $p=0.28$; linear regression) or parallel VA (*Figure 6B*; $R^2=0.12$; $p=0.40$; linear regression).

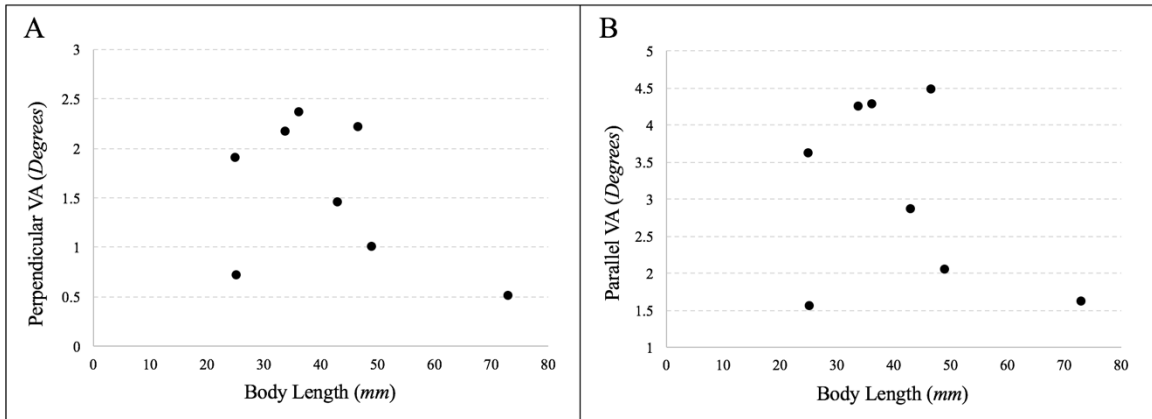


Figure 6: Species length did not correlate with either A) perpendicular VA (linear regression; $R^2=0.19$; $p=0.28$) or B) parallel VA (linear regression; $R^2= 0.12$; $p=0.40$).

Conspecific Spatial Perception Models

Within models of *A. pseudonietana* and *S. equale* perception of conspecific hindwings (*Figure 7*), males had slightly coarser vision than females. Regardless of sex and species, grasshoppers could only resolve pattern differences if hind-wings were 10-25cm from the observer. If hind-wings were 25-50cm from the observer, patterning of the conspecific is lost. Within the model, no spatial perception of conspecifics at 100cm or greater distances is possible.

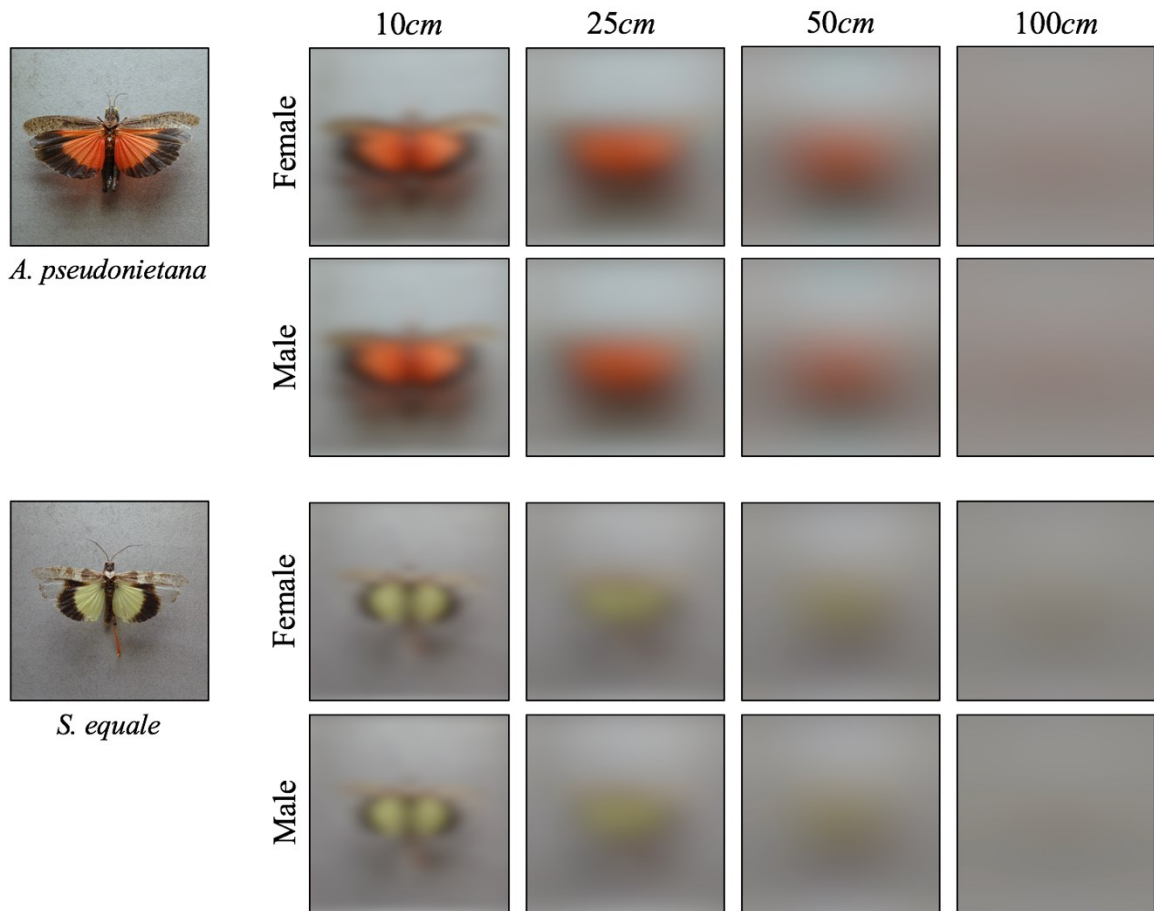


Figure 7: When considering bandwing conspecific VA, hind-wing patterning degrades quickly with distance. The images of *A. pseudonietana* and *S. equale* on the left are unmodified. The upper set of eight images shows *A. pseudonietana* male and female conspecific perception from 10-100cm. The lower set of eight images shows *S. equale* male and female conspecific perception from 10-100cm. Female resolution is finer than that of males within each species, and *A. pseudonietana* resolution is finer than that of *S. equale* overall.

Analysis of Regional Variation in Visual Acuity

Preliminary (n=1 per species and sex combination) whole eye analysis showed possible variation in VA along the surface of the eyes of individual bandwings (*Figures 8-9*).

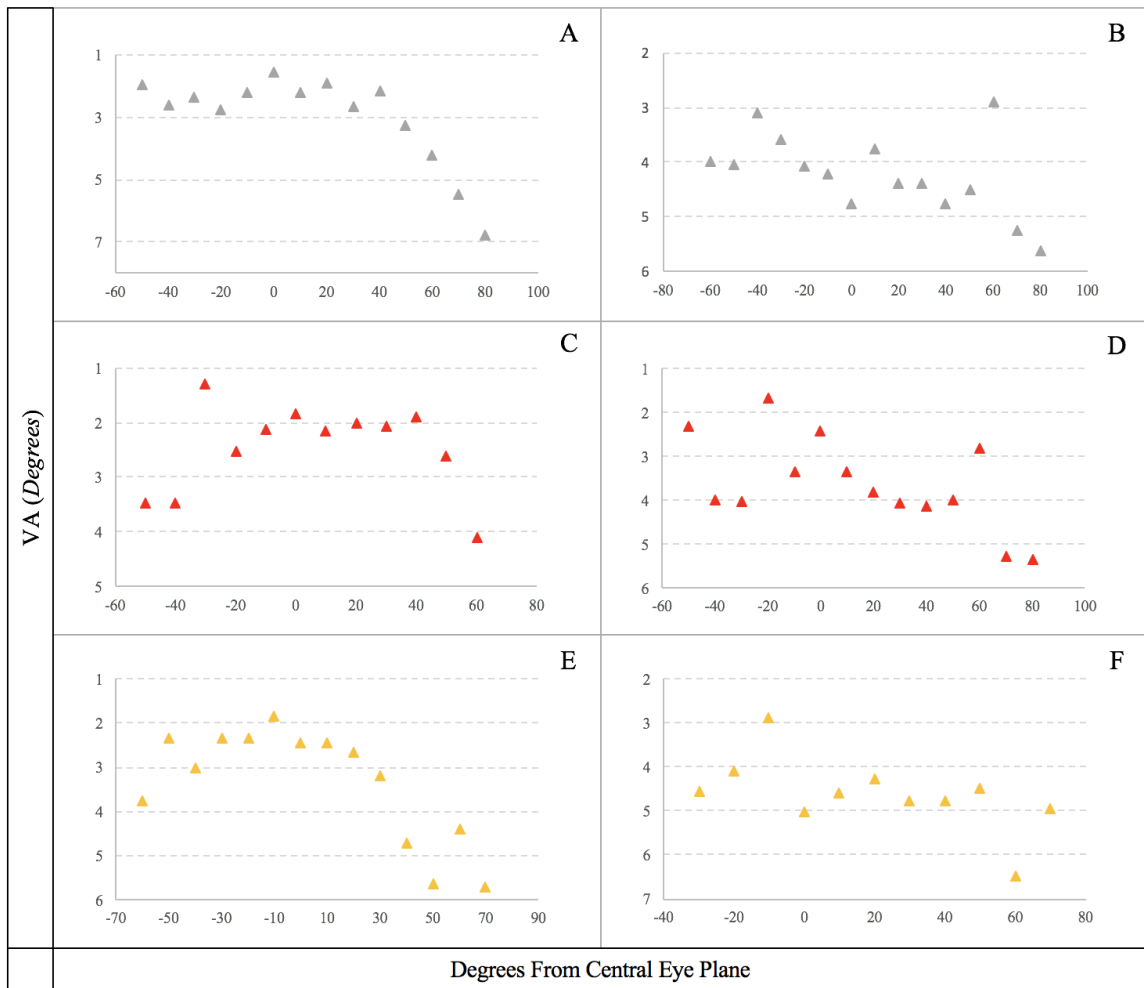


Figure 8: Regional differences in female VA across the eye with A) D. carolina perpendicular VA and B) parallel VA, C) A. pseudonietana perpendicular VA and D) parallel VA, and E) S. equale perpendicular VA and F) parallel VA.

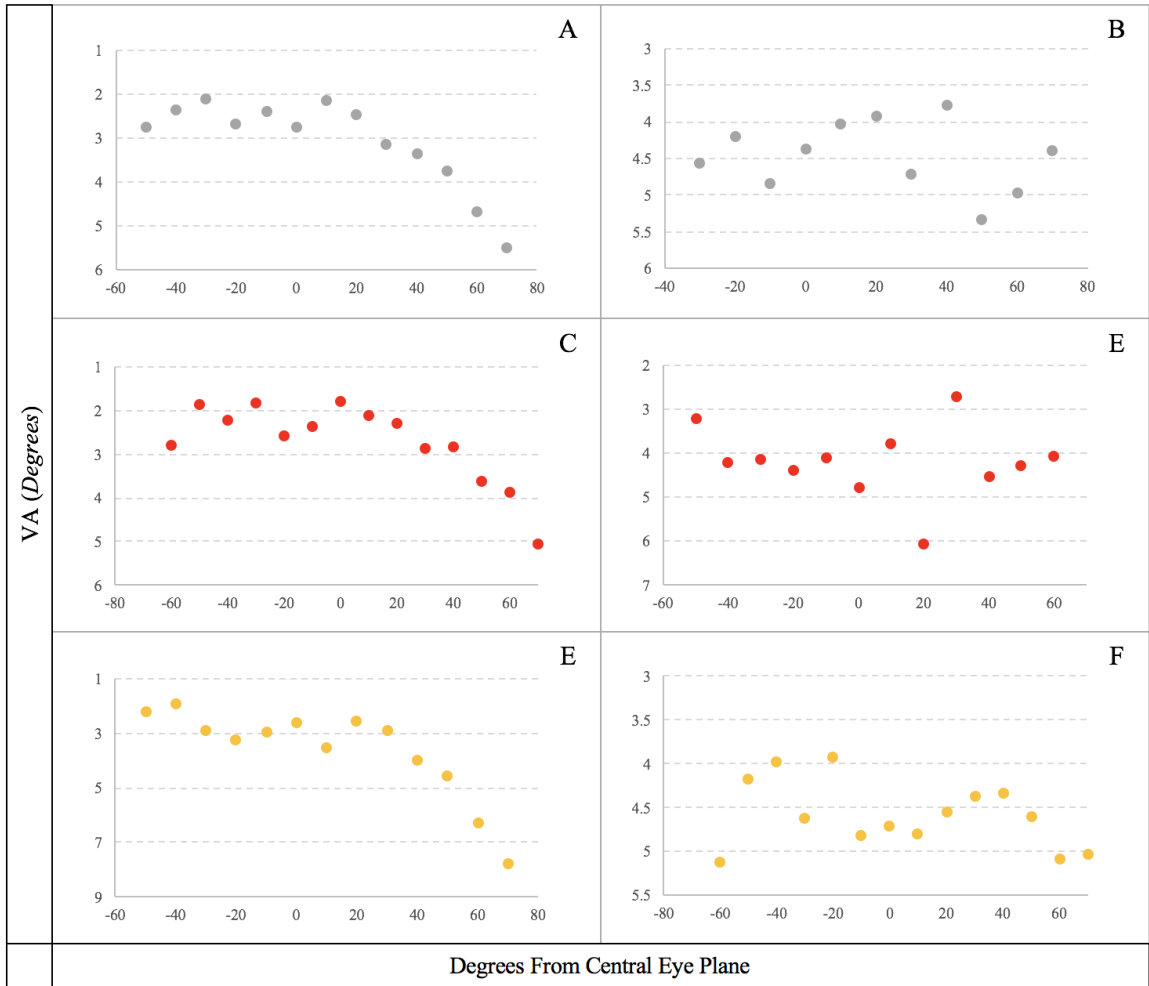


Figure 9: Regional differences in male VA across the eye with A) *D. carolina* perpendicular VA and B) parallel VA, C) *A. pseudonietana* perpendicular VA and D) parallel VA, and E) *S. equale* perpendicular VA and F) parallel VA.

DISCUSSION

Bandwings and Other Grasshopper Species

Our measurements of bandwing perpendicular VA (*Table 2*) are comparable to previously recorded grasshopper values. *L. migratoria* (subfamily Oedipodinae) have an acuity of $\sim 1.8^\circ$ (Horridge 1978), *Schistocerca gregaria* (family Acrididae) has an acuity of $\sim 1.9^\circ$ (Horridge 1978; Krapp and Gabbiani 2005) and *Gryllus bimaculatus* (family Gryllidae), has an acuity of $\sim 2.0^\circ$ (Labhart et al. 2001). Bandwings also have fine VA when compared to most other insects (Land 1997). Animals with this degree of VA do not rely on detailed spatial information, but can visually navigate and use landmarks in their environment (Land 1997; Warrant and Dacke 2011).

In all grasshoppers tested, perpendicular VA was significantly finer than parallel VA (*Table 1*). Non-bandwings had finer average perpendicular VA (5-80% finer) and parallel VA (12-64% finer) than band-winged grasshoppers (*Table 1*). Our data suggest that other grasshopper species, sampled from the same environment as bandwings, have finer spatial vision. When compared to other grasshoppers, bandwings may be less likely to rely on their vision in predator defense (Humphries and Driver 1970; Cott 1940). *Melanoplus*, have shorter predator escape distances than Oedipodinae (Butler 2013). Prolonged movement away from a predator, without erratic protean behavior, might not be beneficial for these species (Humphries and Driver 1970). Grasshoppers without a protean adaptation may need to better resolve predators than bandwings, and therefore more clearly determine when to initiate their escape behavior. To test this hypothesis, and to more clearly

understand differences between bandwing and non-bandwing acuity, non-bandwing acuity and predator avoidance behaviors must be further analyzed.

Bandwing Visual Acuity Perpendicular to the Horizon

Bandwing perpendicular VA was 42-56% finer than parallel VA (*Tables 1-5*). These findings likely result from their non-spherical eye shape. Typically, band-wing eyes are larger perpendicular than parallel the horizon. The eye is then flatter perpendicular to the horizon. The functional significance of this difference is still unknown, but fine perpendicular VA may aid in navigation during flight (Land 1997).

Contrary to findings in other animals (Jander and Jander 2002; Kiltie 2000; Veilleux 2014), body size was not correlated with perpendicular VA (*Table 3; Figure 6A*). These results may suggest that VA is not directly influenced by body size, and therefore eye size.

Table 5: Percent Differences in Bandwing Perpendicular VA and Parallel VA.

Species	Sex	% Finer Perpendicular VA
<i>D. carolina</i>	male	49%
	male	56%
<i>A. pseudonietana</i>	male	47%
	female	55%
<i>S. equale</i>	male	42%
	female	49%

The most parsimonious perpendicular VA model did not include facet diameter (*Table 2*). These results indicate that solely eye curvature, and not facet density, influences differences in perpendicular VA. Unlike other insects, where changes in facet diameter

across the eye surface regionally alter acuity (Perl and Niven 2016), bandwing perpendicular VA is only significantly affected by the curvature of the eye.

S. equale had significantly coarser perpendicular VA than other measured bandwings (Table 3; $p < 0.05$). However, without behavioral observations and modeling, it is difficult to determine whether coarser vision has any significant effect on *S. equale* behavior. These species were collected from the same environment at the same time of year. While VA differences may be due to unknown ecological factors, like microhabitats, the reason and functional relevance of these differences are unclear.

Bandwing Visual Acuity Parallel to the Horizon

Bandwing parallel VA was significantly coarser than perpendicular VA (Table 1, see previous section). Overall, GLMs of parallel VA were not as parsimonious as those of perpendicular VA. Unlike perpendicular VA, the most parsimonious parallel VA model included facet diameter (Table 2; $p < 0.05$). However, given 1) the relatively weak parsimony of the GLM (Table 2), 2) small differences in predicted acuity by facet size (Table 4), and 3) the low measure of fit in regression analysis (Figure 4; $R^2 = 0.11$), these results show facet size to be a small factor influencing parallel VA.

Sex Differences

Females were found to have finer acuity across multiple species (Tables 3-6). Female perpendicular VA was 17-19% finer and parallel VA was 5% finer than their male counterparts (Tables 2-6). This sexual dimorphism in bandwing vision may indicate that

females need finer acuity for specific tasks. As flight behaviors have been related to courtship (Willey and Willey 1969), females may need finer acuity to observe male displays at a distance. Females may also rely more on their camouflage, as they are less active than males (Willey and Willey 1969), and therefore need finer acuity (*Table 2*) to detect predators at greater distances. However, our conspecific perception models (see next section), suggest that this dimorphism may not have behavioral significance and may simply be the result of larger female body size and eye size.

Table 6: Female bandwings have finer VA than males

Species	Visual Axis	% Finer VA in Females
<i>D. carolina</i>	perpendicular	19%
	parallel	5%
<i>A. pseudonietana</i>	perpendicular	19%
	parallel	5%
<i>S. equale</i>	perpendicular	17%
	parallel	5%

Conspecific Spatial Perception Models

Model images of *A. pseudonietana* and *S. equale* perception of conspecific hind-wing patterning yielded similar results in both males and females (*Figure 7*). When compared to unmodified images, bandwing perception of detail in patterning was greatly reduced even at 10cm. Contrast between the large bands was still visible, but the finer aspects of the pattern were not resolved. At 25cm the conspecific was visible, but pattern resolution was virtually non-existent. At 50cm an object was visible, but discerning its attributes became difficult. Finally, at 100cm a bandwing simply resembled the background. Interestingly,

due to translucence on *S. equale* wing tips, its pattern shape was independent of wing shape, possibly aiding in differentiation between conspecifics and other species.

These spatial perception models represent ideal viewing conditions for the image observer, with the viewer at an ideal angle perpendicular to the conspecific wing surface. They do not take movement (Land 1999b), variable light conditions (Kiltie 2000; Warrant and Dacke 2011), bandwing neurological image processing (Mizunami 1995), and environmental obstructions into account. These factors could either have no effect or serve to worsen overall image resolution. Though bandwing visual capabilities are sexually dimorphic, with females having approximately 20% finer vision than males (*Table 6*), and with almost ideal viewing conditions in this model, a receiver likely cannot obtain enough detail from a conspecific's hind-wing pattern to discern mate quality even at the short distance of 10cm. Additionally, grasshoppers are not known to have red-cones (Briscoe and Chittka 2001) so red-winged species, like *A. pseudonietana*, would likely have reduced contrast when viewing conspecific hind-wings when compared to human vision. As little detail can be perceived in conspecifics, these results suggest that while an observer may be able to recognize a conspecific at short distances, hind-wing coloration and patterning likely did not develop as a signal of mate quality. Instead, other signals may be more important to bandwing courtship, like audible clicks or pheromones (Willey and Willey 1969; Otte 1970; Candolin 2003).

Compared to bandwings, predatory birds have finer acuity (0.44° - 0.00625° ; Brandley pers. com.) and better long-wavelength color discrimination (Bennett and Théry 2007). Sexual

dimorphism in hind-wing patterning and coloration was also not observed within bandwings (Salazar Pers. Com.). Together, these factors suggest that hind-wing coloration serves a similar purpose for all individuals within a species. Thus, it is likely that hind-wing coloration and patterning only evolved as a protean predator defense mechanism. This protean effect is achieved by quick changes in movement and appearance, often with sudden bright coloration displays (Humphries and Driver 1970). It is consistent with observed Oedipodinae crepitation behaviors which involve quick jagged movements and the presentation of brightly colored hind-wings (Willey and Willey 1969). These movements may even induce an escape response in predators (Humphries and Driver 1970).

Analysis of Regional Variation in Visual Acuity

Preliminary regional VA analysis showed possible variation in VA across the eye edge in individuals. Within each individual, acuity was finer along the flatter part of the eye, and was coarser in areas that appear to be more curved. On the perpendicular axis, this flat acuity area constituted most of the eye surface (*Figures 8-9*). This area of fine VA roughly aligned with the area of the eye used for sampling in the rest of our study, showing our measurements were at the most acute eye region. More analysis is needed to explore any statistically significant VA trends across the eye surface in band-winged grasshoppers, and how these trends may relate to the ecology or behavior of each species.

Future Directions

We need to more clearly tie our differences in VA to behavior in bandwings, as there are many opportunities to further explore this relationship. In terms of adaptations to predators, more work could be done to validate the protean hypothesis. Field observations to determine whether crepitation behavior is protean, if there is a difference in escape success rate between bandwings and other flying grasshoppers, and if differences in wing coloration have any effect on escape success are all needed to gain a clear understanding of this behavior. To address the possibility of an adaptive sex dimorphism in VA, response to predators could also be experimentally compared between sexes.

Finally, a more detailed phylogenetic analysis of bandwings could help elucidate the evolutionary history of this subfamily. This phylogeny, when compared with recently collected data on hind-wing coloration (Salazar pers. com.), behavioral observations, and VA could suggest causal links to speciation events in Oedipodinae. Such work would put the development of visual systems in a detailed evolutionary context, possibly revealing trends in insects and other animals that are integral to our understanding of their visual ecology.

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Software

Microsoft Excel for Mac 2017; 15.32; Microsoft; Santa Rosa, CA

Image J; 1.50i; National Institutes of Health; Bethesda, MD

R: A language and environment for statistical computing; 2013; R Core Team

Equipment

AmScopeX for Mac MU; MW Series 05/26/2016; AmScope; Irvine, CA

LED312DS; Fotodiox; Gurnee, IL

M28Z Zoom Stereo Binocular Microscope; Swift; Carlsbad, CA

14MP USB 3.0 Real-Time Live Video Microscope USB Digital Camera; AmScope; Irvine, CA