

Ultimate Causes and Consequences of Coloration in North American Black Widows

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
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ABSTRACT

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Abstract

Researchers have long assumed that black widow coloration functions as a warning signal to avian predators. However adult female black widow coloration does not resemble typical warning coloration in two distinct ways. First, black widows are less colorful than most other documented aposematic species. Second, the hourglass shape of an adult female varies both between species and within a site. Here I examine the ultimate causes and consequences of North American black widow coloration.

In chapter two I present data that suggest that black widow coloration not only functions as an aposematic signal to avian predators, but has also been selected to be inconspicuous to insect prey. In choice experiments with wild birds, I found that the red-and-black coloration of black widows deters potential predators: wild birds were ~3 times less likely to attack a black widow model with a red hourglass than one without. Using visual-system appropriate models, I also found that a black widow's red-and-black color combo is more apparent to a typical bird than typical insect (Euclidean color distance ~2.2 times greater for birds than insects). Additionally, an ancestral reconstruction revealed that red dorsal coloration is ancestral in black widows and that at some point some North American black widows lost their red dorsal coloration (while maintaining the ventral hourglass). Behaviorally, differences in red dorsal coloration between two North American species are accompanied by differences in microhabitat

that affects how often a bird will view a black widow's dorsal region. All observations are consistent with a cost-benefit tradeoff of being conspicuous to potential predators while being inconspicuous to prey. I suggest that avoiding detection by prey --- combined with Müllerian mimicry --- may help explain why red-and-black aposematic signals occur frequently in nature.

In chapter three, I examine the variation in hourglass shape. Classical aposematic theory predicts near uniformity in warning signal appearance because a uniform signal is easier to learn to avoid than a variable signal. However the shape of the hourglass of North American black widows appears to vary both within and between sites in ways that are inconsistent with classical aposematic theory. Using 133 black widows of three different species from nine sites across the United States, I quantified the variation in hourglass shape and examined how Müllerian mimicry, species type, and condition each influenced hourglass shape. A principal components analysis revealed that 84.5% of the variation in hourglass shape can be explained by principal components 1, 2, and 3, which corresponded to hourglass size (PC1), the separation between hourglass parts (PC2), and the slenderness of the hourglass (PC3). Both a black widow's condition and species significantly predicted hourglass shape; however I found no support for localized Müllerian mimicry within different geographical regions. My results suggest a relaxed role for selection on hourglass shape. I discuss several hypotheses that could explain the variation in hourglass morphology including that potential predators may avoid any red markings rather than an exact shape (categorical rather than continuous perception).

In chapter four I expand on my work from chapter two to examine the eavesdropper's perspective on private communication channels. Private communication may benefit signalers by reducing the costs imposed by potential eavesdroppers such as parasites, predators, prey, or rivals. It is likely that private communication channels are influenced by the evolution of signalers, intended receivers, and potential eavesdroppers, but most studies only examine how private communication benefits signalers. Here, I address this shortcoming by examining visual private communication from a potential eavesdropper's perspective. Specifically, I ask if a signaler would face fitness consequences if a potential eavesdropper could detect its signal more clearly. By integrating studies on private communication with those on the evolution of vision, I suggest that published studies find few taxon-based constraints that could keep potential eavesdroppers from detecting most hypothesized forms of visual private communication. However, I find that private signals may persist over evolutionary time if the benefits of detecting a particular signal do not outweigh the functional costs a potential eavesdropper would suffer from evolving the ability to detect it.

Dedication

To Hambone and the guys who made the He-man heyeayea song.

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1. Introduction and study species

1.1 Introduction

Warning signals (aposematic signals) are among some of the most striking uses of coloration found in the natural world (Poulton 1890, Cott 1940, Ruxton, Sherratt et al. 2004). Unlike color patterns caused by sexual selection (Darwin 1871, Andersson 1994), warning signals benefit the signaler via natural selection: if an individual advertises its defenses (or unpleasantness) through bright or high-contrast coloration patterns, predators may be less likely to attack because they can learn to avoid those color patterns. Examples of warning signals have been found in numerous taxa including bees (Plowright and Owen 1980), beetles (Jones 1932), brittle-stars (Grober 1988), butterflies (Brower 1958), frogs (Fritz, Rand et al. 1981), salamanders (Brodie and Brodie 1980), skunks (Lariviere and Messier 1996) and snakes (Brodie 1993).

Black widow spiders (genus *Latrodectus*) have long been thought to possess aposematic red-and-black coloration (e.g. Vamosi, Przeczek et al. 2008). However, in many ways the coloration of adult female North American black widows (Figure 1) seems to be different than many other documented types of warning coloration. First, black widows (see study species section) are less colorful than most other documented aposematic species. While many warning signals consist of predominantly red coloration with black accents, black widows are the reverse with a predominantly black coloration with red accents (Figure 2). Second, adult female North American black

widows are also less colorful than both their juvenile forms and other species of *Latrodectus*, and thus seem to represent a reduction in a warning signal (D'Amour, Becker et al. 1936, Levi 1959, McCrone and Levi 1966, Kaston 1970). Lastly, the hourglass shape of an adult female varies both between species and within a site (Levi 1959, Kaston 1970) despite that classical aposematic theory suggests that warning signals should be near uniform (see chapter 2).

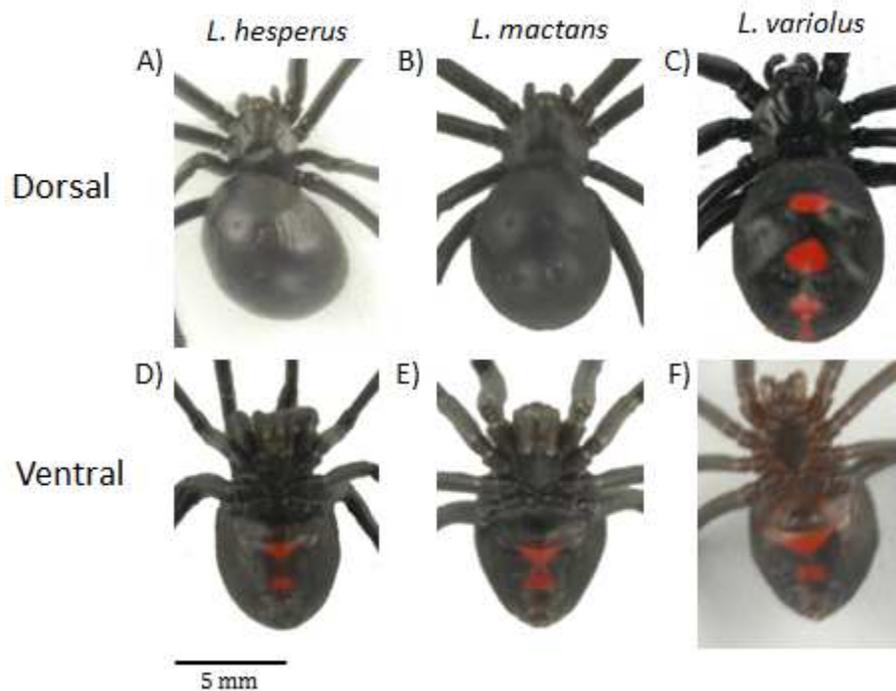


Figure 1: Typical dorsal (A-C) and ventral (D-F) patterning of three species of North American *Latrodectus*. Note that black widows typically position themselves so that the ventral hourglass faces upwards. While all three species have a ventral red hourglass, only *L. variolus* typically has red dorsal spots. While these photos represent a typical individual, ventral patterning can vary greatly between populations and individuals of the same species.

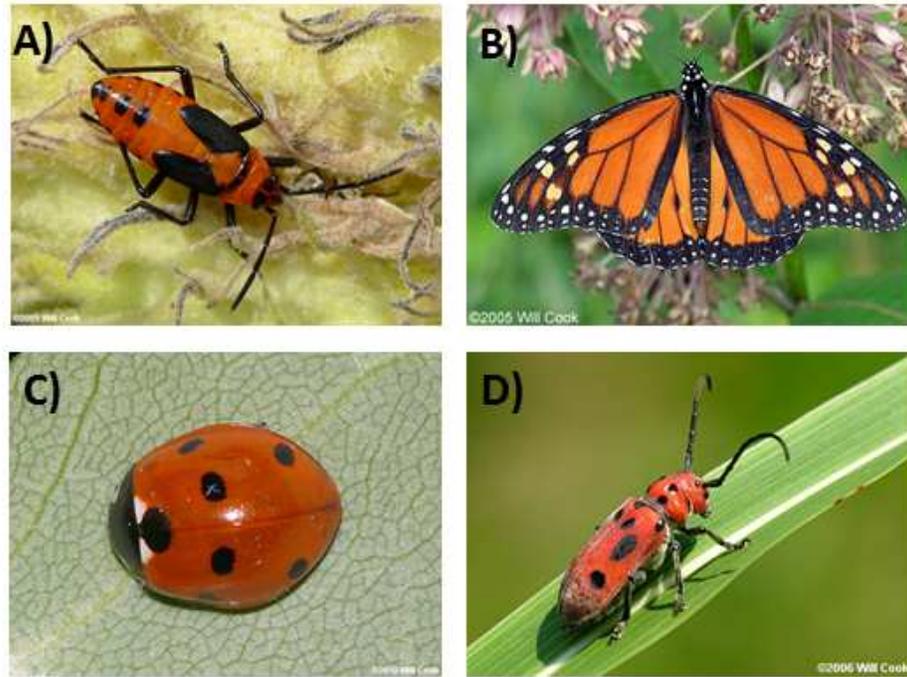


Figure 2: Common North American aposematic insects that utilize long wavelength warning coloration. Note that the allocation of colors is the opposite of black widow spiders, with primarily red/orange coloration with black accents. All insects were photographed in Durham County, N.C. A) The small milkweed bug (*Lygaeus kalmia*). B) The monarch butterfly (*Danaus plexippus*). C) The seven-spotted ladybug (*Coccinella septempunctata*). D) The red milkweed beetle (*Tetraopes tetraophthalmus*). All photos property of Will Cook and used with permission.

This thesis attempts to explain the unique color patterning of adult female North American black widows. In chapter 2, I argue that selection on black widow coloration has served to maximize conspicuousness to predators as a warning signal while simultaneously reducing detection by black widow prey. In chapter 3, I examine the natural variation in hourglass shape between three species and nine sites while trying to determine what ultimate forces cause variation in hourglass shape. In chapter 4 I examine private communication channels (including black widow coloration) from the

perspective of the potential eavesdropper to ask if functional costs or taxon-specific evolutionary constraints may keep private communication channels secret over evolutionary time frames.

1.2 Study species

There are three species of black widow found in North America: the western black widow (*Latrodectus hesperus*), the southern black widow (*Latrodectus mactans*), and the northern black widow (*Latrodectus variolus*). Together the three species make a paraphyletic group within the genus *Latrodectus* (Garb, Gonzalez et al. 2004) which in turn lies within the cobweb spider family (*Theridiidae*). The North American black widows' closest relative is the red widow (*Latrodectus bishop*), which is believed to be the sister species to *L. variolus* (Garb, Gonzalez et al. 2004). Other members of the genus are found in all continents except Antarctica (Levi 1959, Garb, Gonzalez et al. 2004) and include the redback spider (*Latrodectus hasselti*) in Australia and the white widow (*Latrodectus pallidusi*) in the Middle East.

North American black widows are primarily sit-and-wait predators that rely on prey hitting their disorganized cobweb (Blackledge, Summers et al. 2005, Blackledge and Zevenbergen 2007). Black widows usually sit upside down in their web with their ventral surface (and hourglass) pointing upwards. While their venom is capable of killing vertebrates including humans (Maroli, Bettini et al. 1973), human bites from black widows are actually relatively rare because black widow fangs are usually unable to

pierce human skin. Instead their venom often serves a predatory function and has components designed to subdue a variety of prey including insects and crustaceans (Haney, Ayoub et al. 2014). Black widow diet varies by location, but often consists of cursorial insects (Hódar and Sánchez-Piñero 2002, Salomon 2011). Predation events on black widows are rarely observed, but birds, mammals and reptiles may exert predation pressure on black widow spiders (Levi 1959, Hodar and Sanchez-Pinero 2002).

It is unknown what pigments cause the red-and-black coloration in female black widow spiders, however, most red coloration in spiders is caused by ommochromes (Foelix 2011). North American black widows develop over a period of 7 instars, and juvenile coloration ---while often more colorful than the adult form--- appears to vary by location (McCrone and Levi 1966). Male black widows are ~10% the size of female black widows (Kaston 1970).

Sexual selection varies immensely over the genus *Latrodectus*. Male redback spiders exhibit adaptive self-sacrifice towards cannibalizing females; it is believed that they are functionally sterile once they reach a female's web, largely because they are highly unlikely to be able to reach another female's web (Andrade 1996, Andrade 2003, Stoltz and Andrade 2010). However *L. hesperus* males actually exhibit male-mate choice and prefer to mate with large virgin females over small non-virgins (MacLeod and Andrade 2014). Within the North American black widows, sexual selection is believed to

occur through both olfactory and vibrational cues, with little role for vision (Ross and Smith 1979, Johnson, Trubl et al. 2011, MacLeod and Andrade 2014).

Black widow vision has not been studied. They exhibit the common 4-4 eye pattern observed across Theridiidae (Foelix 2011), however their eyes are not very large (diameter of ~0.2 mm). This limited eye size makes it unlikely that black widows have good spatial resolution (Johnsen 2012, Land and Nilsson 2012), especially considering that many nocturnal animals sacrifice spatial resolution for sensitivity (Warrant 2008). As a result black widow coloration has probably been shaped by factors other than black widow vision.

2. Receiver-dependent signaling: red-and-black aposematic signals in North American black widows are both conspicuous to predators and cryptic to prey

2.1 Introduction

Well-defended animals may benefit if they signal to predators that they are dangerous or otherwise distasteful (Cott 1940, Ruxton, Sherratt et al. 2004). As a result, many animals with defenses have aposematic (warning) signals that are conspicuous to many species (Jones 1932, Smith 1975, Caldwell and Rubinoff 1983, Sillentullberg 1985). However, not all aposematic signals are equally effective at deterring attack; numerous studies have suggested that specific characteristics (*e.g.* contrast, hue, modality, and patterning) of an aposematic signal can influence predator avoidance and memory (Gittleman and Harvey 1980, Sillentullberg 1985, Roper and Redston 1987, Roper and Cook 1989, Rowe and Guilford 1999, Zylinski and Osorio 2013). Fewer studies have examined how such characteristics might affect the behavior of viewers other than predators. The same patterns that serve as aposematic signals may also have to attract mates (Maan and Cummings 2009), communicate with rivals (Crothers, Gering et al. 2011, Crothers, Gering et al. 2012), or maintain crypsis against prey. Aposematic phenotypes with characteristics that satisfy multiple functions may grant a selective advantage to the signaler similar to those seen in other signals that satisfy multiple functions (*e.g.* Endler 1991, Marshall 2000, Cummings, Rosenthal et al. 2003, Stuart-Fox and Moussalli 2009).

One potentially strong selective pressure that could shape the characteristics of aposematic signals is foraging success; a conspicuous aposematic signal could lower the foraging rate of the signaler if it is used by potential prey to avoid the signaler. Therefore, while selection favors aposematic signals that are conspicuous to predators, selection should also favor aposematic signals that are inconspicuous to prey. Differences in sensory physiology and behavior between predators and prey may make it possible to satisfy both selective pressures simultaneously. For example, if predators and prey differ in their sensory physiology, individuals could signal with characteristics that are more conspicuous to the former than to the latter (e.g. Endler 1983, Cummings, Rosenthal et al. 2003, Mathger, Shashar et al. 2009, Brandley, Speiser et al. 2013). Many non-aposematic signalers may use extreme-wavelength (ultraviolet or red) or polarized signals as private communication channels in cases where potential eavesdroppers (such as prey) lack the ability to detect such signals (Brandley, Speiser et al. 2013). Additionally, if predators and prey generally view signalers from different angles, signalers could communicate in ways that are more conspicuous from typical predator viewing angles than from typical prey viewing angles (Marshall 2000, Cummings, Jordao et al. 2008). For example, the non-aposematic fiddler crab *Uca tangeri* uses differences in viewing angles to be conspicuous when viewed laterally by conspecifics yet maintain crypsis when viewed from above by avian predators (Cummings, Jordao et al. 2008).

American black widow spiders (genus *Latrodectus*), specifically the adult females, are well-suited for studying the evolution of multi-functional aposematic signals that remain conspicuous to predators but are inconspicuous to prey. Adult female black widows may have up to two different types of red markings: all species possess the iconic ventral red hourglass, while some species also exhibit red dorsal red markings (Figure 1; Kaston 1970). Although prior experimental data are lacking, the red-and-black coloration has long been assumed to function as an aposematic signal (e.g. Vamosi, Przeczek et al. 2008) in part because adult female black widows are well-defended against their vertebrate predators by both their secreted defensive web globules (Vetter 1980) and their venomous bite (Maroli, Bettini et al. 1972). When black widows move along their webs, they typically present their ventral hourglass upwards (Blackledge, Summers et al. 2005, Blackledge and Zevenbergen 2007). The presentation of the hourglass away from the ground suggests that black widows may be trying to warn off birds. Birds often feed on spiders because of their nutritional advantage over insects (Jansson and von Brömssen 1981, Hodar and Sanchez-Pinero 2002, Ramsay and Houston 2003, Arnold, Ramsay et al. 2007), so a conspicuous aposematic signal towards birds might benefit black widows. However black widows are also sit-and-wait predators (Kaston 1970), and conspicuousness may deter their prey [typically insects: (Kaston 1970, Hodar and Sanchez-Pinero 2002, Salomon 2011)].

Differences between avian predators and insect prey in spectral sensitivity and viewing angle could allow black widows to maintain a conspicuous aposematic signal to avian predators while adjusting certain characteristics to minimize the signal's conspicuousness to prey. Avian predators and insect prey differ consistently in their visual physiology. Birds typically have a long-wavelength sensitive photoreceptor that most insects lack (Briscoe and Chittka 2001, Hart 2001, Hart and Vorobyev 2005, Frentiu, Bernard et al. 2007). If black widows use a hue of red that minimizes non-red reflectance (including reflectance in the ultraviolet), the red markings on a black background should be more conspicuous to birds than to nearly all insects. Birds and insects also differ in the angle from which they typically view black widows; birds may approach black widows from any direction while the web of the black widow is specifically designed to catch insects approaching from below (Blackledge, Summers et al. 2005, Blackledge and Zevenbergen 2007). If some species of black widows reside in microhabitats where birds are unlikely to approach black widows from below, these species of black widows could limit their red coloration only to the directions from which birds are likely to approach.

Here, we examine how both avian predators and insect prey may have influenced the coloration of black widows through an integrative approach that combines behavioral experiments, simulations of visual contrast, the reconstruction of ancestral states, and comparisons between species. First, to examine whether the coloration of a black widow deters predators, we present painted models of black

widows (with and without a red hourglass) to wild songbirds. We then use simulations of visual contrast to determine whether the specific red and black hues used by black widows are more obvious to avian predators than insect prey. We then turn our attention to the differences in dorsal coloration between species of black widows. To determine the evolutionary history of red dorsal coloration, we estimate the ancestral state of dorsal coloration at key nodes on a molecular phylogeny. To examine if differences in dorsal coloration may have been influenced by differences in typical viewing angles of predators and prey (which vary with microhabitat) we then compare the microhabitat of two species of sympatric black widows that differ in dorsal coloration. Taken together our data suggest that the visual systems of insect prey have influenced the characteristics of the black widow's aposematic signal. We suggest that potential eavesdroppers (such as prey) may be an underappreciated selective force on the evolution of aposematic signals, and that differences in sensory physiology between avian predators and insect prey may help explain the common co-occurrence of aposematic signals that pair long-wavelength (*i.e.* yellow, orange, and red) and black coloration.

2.2 Presentations of black widow models to birds

2.2.1 Methods

In order to determine whether the red-and-black coloration of black widows deters avian predators, we purchased a digital rendering of a 3-D black widow model

that closely resembled *L. mactans* (abdomen length = 0.9cm, cephalothorax length = 0.5 cm; TurboSquid Inc. New Orleans, LA, USA). We then used the rendering to print non-toxic black widow models using a 3-D printer (vero blue; C.Ideas, Crystal Lake, IL, USA). Each black widow model was then hand-painted either all black (all-black model), or black with a ventral red hourglass (hourglass model) using acrylic paint that approximately matched the spectral reflectance of real spiders (Figure 16 in Appendix A; black, Heavy Body Mars Black, Liquitex Artist Materials, Piscataway, NJ, USA; red, Berry Red, DecoArt, Stanford, KY, USA).

To attract wild birds for the experimental model presentations, seven different locations throughout Durham County, NC, were stocked daily with seed. To ensure that the color of seeds would not bias a bird's future response to black widow models, each location received either black oil sunflower seeds (black color) or safflower seeds (white color) in subsequent seasons. At the time of presentation, all food was removed and a black widow model (all-black model or hourglass model) was placed with its ventral side up on a circular platform feeder (radius = 8.9 cm; Songbird Bowl, Kaytee Products Inc. Chilton, WI, USA). To limit the effects of a bird's prior experience and the location of a site, each site received both an all-black model and an hourglass model at least a week apart and the first black widow model type presented altered between sites. Only time periods lacking rain and with mild/no wind (<10 MPH) were used for presentations.

The response of birds to each model type was categorized as either landing on the feeder without touching the black widow model (avoid), or touching the model with the beak or foot (attack). Since birds were not banded, only those that we could guarantee were distinct individuals were used for this study; usually this limited the presentations to the first bird of each species at each site, which included Carolina chickadees (*Poecile carolinensis*; n= 18), northern cardinals (*Cardinalis cardinalis*; n= 16), house finches (*Haemorhous mexicanus* n=30), tufted titmice (*Baeolophus bicolor*; n=13), white breasted nuthatches (*Sitta carolinensis*; n= 5), and two species of woodpeckers (*Melanerpes erythrocephalus* and *Dryocopus pileatus*; n = 1 each). However, occasionally we tested more than one individual of a single species at each site if we could distinguish them based on significant differences in plumage.

The responses of birds to our two different models of black widows were analyzed in R using a generalized linear model (glm in the LME4 package). We used both the Akaike (AIC) and Bayesian information criterion (BIC) to find the most parsimonious generalized linear model. Because our initial results suggested that beak size may play a role in the tendency to attack a model black widow, we ran two different analyses. The first analysis looked at bird species (Figure 3), while the second analysis (Figure 4) replaced the bird species variable with the average beak size of each species (Table 7 in Appendix A).

2.2.2 Results

When we controlled for bird (predator) species, we found that wild birds were 2.9 times more likely to attack black widow models that were all-black than black widow models with a red hourglass (Figure 3; $p < 0.01$; glm). Analyses using AIC and BIC both agreed that the most parsimonious generalized linear model examined the response of the birds to different black widow models while controlling for bird species and did not include the effects of prior seed type, site, or interactions between variables. Despite small sample sizes, certain species of birds differed in how aggressive they were toward both the all-black and the hourglass models ($p < 0.05$; glm). The generalized linear model indicated that house finches were likely the least aggressive bird (9% attack rate, $n=32$), while woodpeckers (100% attack rate, $n=2$) were the most likely to attack. When we replaced the bird species variable with an average beak size measurement (Table 7 in Appendix A), we found that beak size was a strong predictor of attack likelihood for both the red hourglass and all-black model (Figure 4; $p < 0.001$; glm).

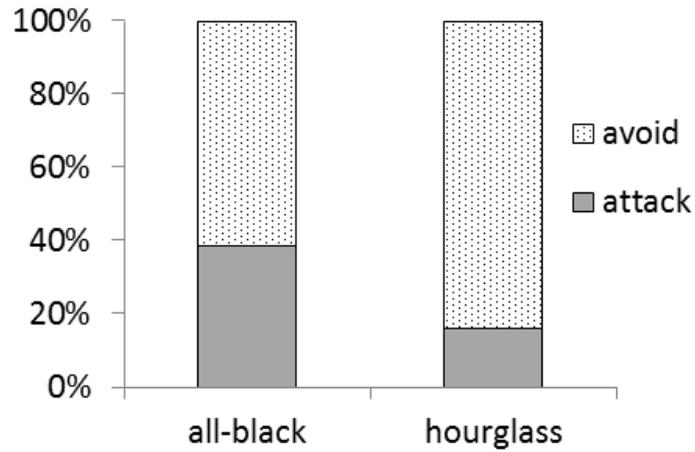


Figure 3: When controlling for bird species, wild birds are more ~3 times more likely to attack a black widow model with an hourglass than one without ($p < 0.01$; generalized linear model.)

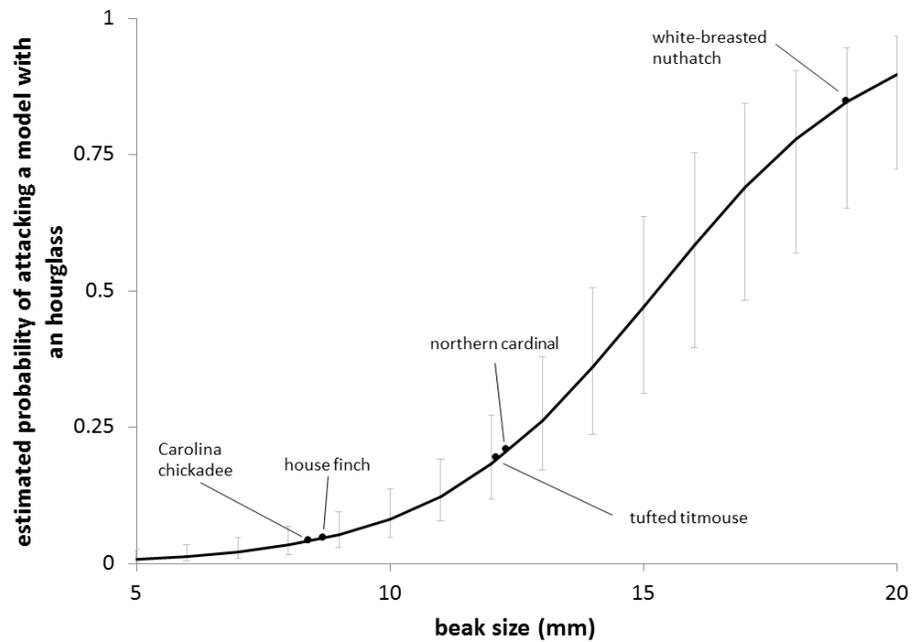


Figure 4: Birds with longer bills are more likely to attack a black widow model with an hourglass ($p < 0.001$; generalized linear model; grey error bars indicate one SEM.) Not shown on the graph are the woodpeckers (average bill length 37 mm, estimated probability of attack = 1)

2.3 Reflectance measurements and visual modeling of black widow coloration

2.3.1 Methods

Because birds and insects differ in the typical spectral sensitivities of their visual systems (Briscoe and Chittka 2001, Hart and Vorobyev 2005), we measured the reflectance of the red markings of adult female black widow spiders to determine if black widow coloration is more conspicuous to avian predators than insect prey. We took reflectance measurements of the black widows *L. mactans* and *L. variolus* using a 200 um fiber coupled with a USB2000 spectroradiometer (Ocean Optics Inc. Dunedin, FL, USA). The incident light (LS-1_CAL calibrated light source; Ocean Optics Inc. Dunedin, FL, USA) was perpendicular to the plane of the spider and the probe was at an angle of 45° and almost touching the spider. Three to five measurements were taken for the hourglass and a nearby black region on the body of each spider (N=14).

Because most insects lack the long-wavelength photoreceptor used in red vision (Briscoe and Chittka 2001), the hourglass of the black widow will contrast most against the black body of the spider in the insect's green color channel. Therefore, we simulated photon catches for the longest wavelength photoreceptor of both an insect ($\lambda_{\max} = 520$ nm; Briscoe and Chittka 2001) and a bird ($\lambda_{\max} = 607$ nm; the blue tit *Cyanistes caeruleus*; Hart and Vorobyev 2005) using the equation:

$$\text{Photon catch} = C \int_{350}^{700} R(\lambda) I(\lambda) S(\lambda) d\lambda$$

where R is the measured spectral reflectance of the black widows, I is the reference solar spectral irradiance (National Renewable Energy Laboratory), S is the spectral sensitivity of the photoreceptor in question, and C is a constant that is independent of wavelength and factors out when different photoreceptors are compared. For the typical spectral sensitivity of the green channel in an insect ($\lambda_{\max} = 520 \text{ nm}$) we used the Govardovskii et al visual pigment template (2000) with the rhabdom length of a field cricket (*Gryllus bimaculatus*; Sakura, Takasuga et al. 2003), and for spectral sensitivity of the red channel in a bird ($\lambda_{\max} = 607 \text{ nm}$) we used a blue tit (*Parus caeruleus*) template that accounts for the spectral filtering by the colored oil droplets found in bird photoreceptors (Hart and Vorobyev 2005). We then calculated the Weber contrast (C) of the red-and-black coloration for the longest wavelength photoreceptor for both a bird and insect where:

$$C = \frac{\text{Photon catch}_{\text{hourglass}} - \text{Photon catch}_{\text{black}}}{\text{Photon catch}_{\text{black}}}$$

In addition to the Weber contrast of the longest wavelength channel, we also calculated the Euclidean color distance as a non-noise limited model of animal vision. Note that while a noise-limited model of animal vision would be ideal (Vorobyev and Osorio 1998, Vorobyev, Brandt et al. 2001), we chose to use a non-noise limited model of animal vision because of numerous problems with applying a noise-limited model to black widow coloration (see Appendix B for further discussion). We calculated the Euclidean color distance of the hourglass from the black abdomen of each widow using two Maxwell triangles corrected for color constancy with the von Kries coefficient law

(see Balkenius and Kelber 2004 for full methods) against the illuminant of a reference solar spectral irradiance (National Renewable Energy Laboratory). For the insect Maxwell triangle, we used a UV, blue and green photoreceptor with lambda maxes at 350 nm, 450 nm, and 520 nm respectively. For the bird Maxwell triangle, we used a blue, green, and red photoreceptor with lambda maxes at 450 nm, 550 nm, and 606 nm respectively (Hart and Vorobyev 2005). Although blue tits do have a UV sensitive photoreceptor, because the reflectance of black widows has little UV reflectance the UV photon-catch should remain relatively constant and should not greatly affect the resulting color distance. By excluding the bird UV photoreceptor, we can compare both Euclidean distances in 2D space, where as if we included the UV photoreceptor comparisons between bird color space (3D) and insect color space (2D) would not be possible.

2.3.2 Results

The red hourglass markings from adult female black widows have minimal reflectance below 550 nm (Figure 5). Notably, adult female black widows have no significant ultraviolet reflectance. We found significant differences in the Euclidean color distance of the hourglass from the abdomen when viewed by birds or insects. A typical bird had a Euclidean color distance between the red and black regions (0.40 ± 0.022 SEM) that was around double that of a typical insect viewing the same regions (0.18 ± 0.025 ; $p < 0.001$; Figure 6). The differences in Euclidean color distance resulted from differences

in the long-wavelength channel of each animal. Photon catch models suggest that the Weber contrast of the hourglass against its black background is ~3.5 times greater ($p < 0.001$) when viewed by a typical bird long-wavelength photoreceptor [median = 4.69; SEM = 1.4; $\lambda_{\max} = 607$ nm (Hart and Vorobyev 2005)] than a typical insect long-wavelength photoreceptor [median = 1.39; SEM = 0.7; $\lambda_{\max} = 520$ nm (Briscoe and Chittka 2001)].

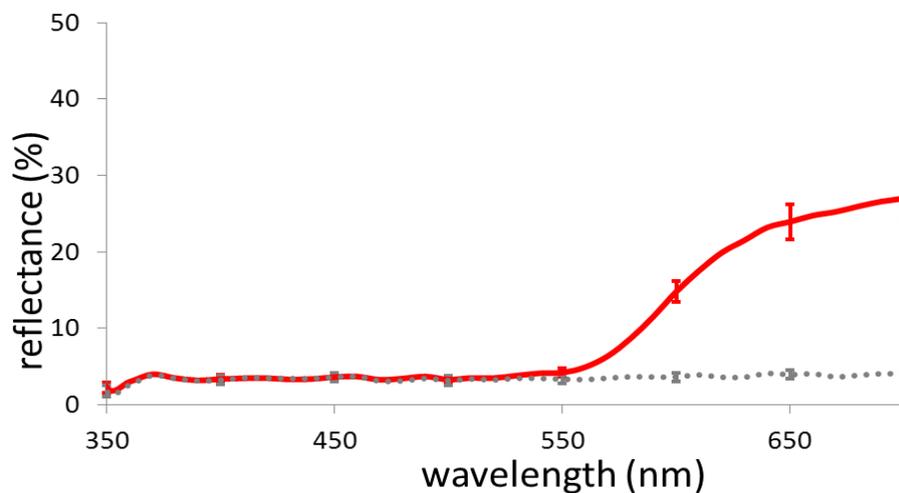


Figure 5: Combined spectral reflectance of *L. mactans* (n=9) and *L. variolus* (n=5). The error bars show \pm the standard error. Solid red line= hourglass, dotted grey = ventral black of the spider. The hourglass has very little reflectance below ~ 550nm, and includes no significant UV reflectance.

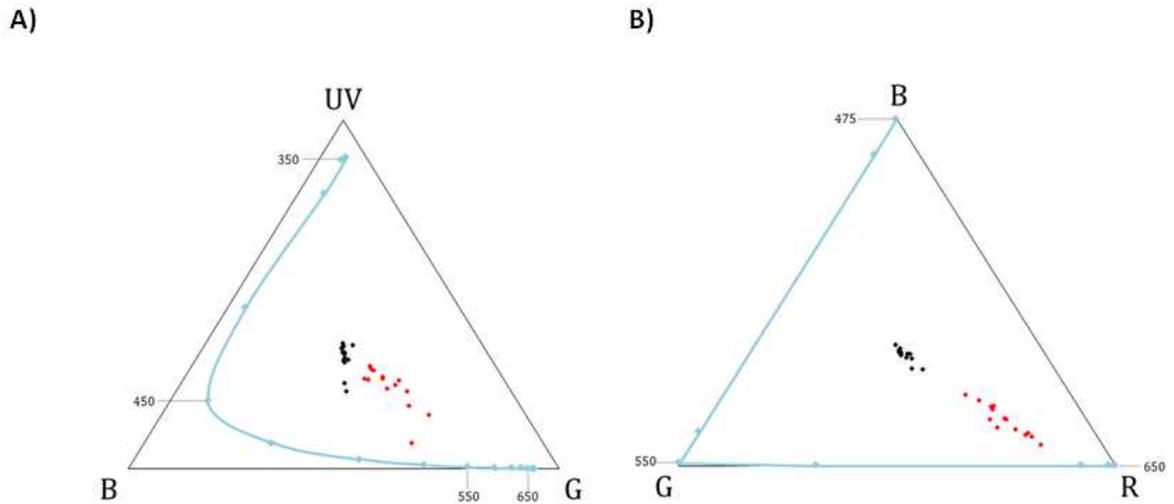


Figure 6: Color-constancy Maxwell triangles for a typical insect (A) and a typical bird (B) viewing 14 individual black widows. The black markers represent the color of the black abdomen, while the red markers represent the color of the red hourglass. Note that that red markers are generally farther away from the black markers in the bird's view (mean color distance = 0.40, standard error = 0.022) than the insect's view (mean color distance = 0.18, standard error = 0.025). See methods for further information on the photoreceptors used.

2.4 Ancestral reconstruction of black widow dorsal coloration

2.4.1 Methods

To infer past selective pressures on black widow coloration, we used the *Lactrodectus* phylogeny from Garbs et al. (2004) so that we could estimate dorsal coloration characteristics at key ancestral nodes. Mitochondrial gene cytochrome oxidase I sequences deposited by Garb et al. (2004) were downloaded from GenBank for species in the *L. mactans* group. The sequences were then aligned using the default settings in MUSCLE (v 3.8.31, Edgar 2004) and then adjusted by eye. A maximum likelihood phylogeny was reconstructed using the GARLI (v 0.95 Zwickl 2006) general time-

reversible model with a proportion of invariable sites and rates gamma distributed among four rate categories (GTR+I+G).

Ancestral state reconstruction was accomplished using the “ace” function in the R package “ape” (Paradis, Claude et al. 2004). This function is a maximum likelihood method for determining the marginal likelihoods of each character state at each node (Pagel 1994). All changes (i.e. red to black, red to white, black to red, etc.) were considered equally likely, with no penalty for reversal.

2.4.2 Results

The ancestral reconstruction of dorsal coloration suggests that the ancestral black widow had red dorsal coloration (marginal likelihood = 1.0), which was subsequently lost in the North American widows *L. hesperus* and *L. mactans* (Figure 7). The common ancestor of all North American widows was twice as likely to have been dorsally all-black (marginal likelihood = 0.66) than having some red dorsal coloration (marginal likelihood = 0.33).

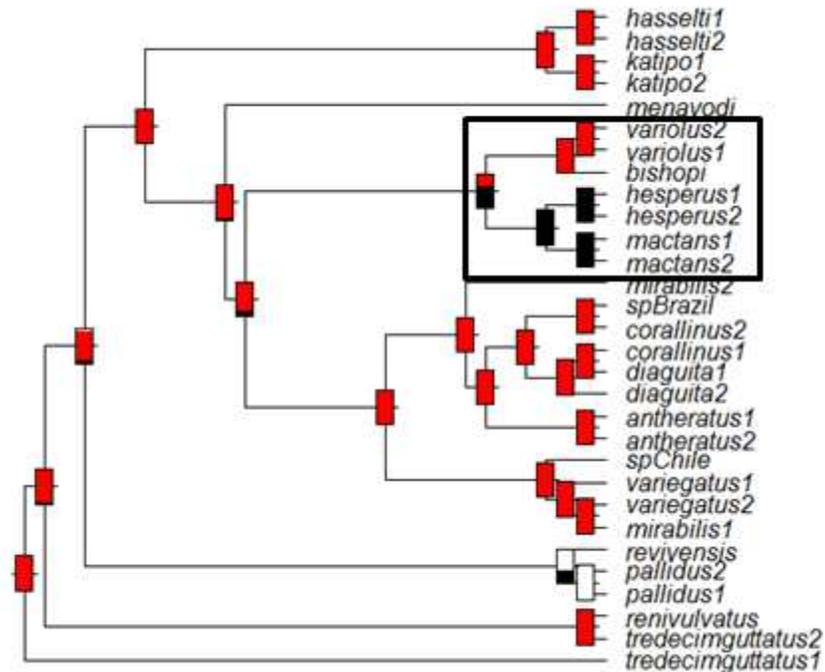


Figure 7: Ancestral reconstruction of dorsal coloration within adult female black widows. The black box indicates the North American species. It appears that an ancestor of the North American widows lost its red dorsal coloration, but when this occurred is not known. Mixed color bars at each ancestral branch indicate the probability that the ancestor had the trait in question. Black bars indicates less than 5% red dorsal coloration on a typical adult female widow of each species, red bars indicate at least 5% dorsal red coloration, and white indicates a pale primary coloration. Phylogeny based on mitochondrial DNA information deposited by Garb et al. (2004).

2.5 Microhabitat choice

2.5.1 Methods

Black widows are more likely to have predators that approach from below --- and therefore need a dorsal aposematic signal --- if they are found at least a few feet above ground level rather than right at ground level. Therefore, we performed laboratory microhabitat experiments using two sympatric species of black widows to

examine if differences in dorsal coloration were consistent with differences in microhabitat preferences. Adult female Northern (*L. variolus*; n = 27) and Southern (*L. mactans*; n = 20) black widows were collected in Liberty County, FL (30.23° N, 84.89° W) and Lee County, FL (26.58° N, 81.92° W), respectively. In the laboratory, widows were then housed in clear plastic boxes (10 x 10 x 10 cm; AMAC Plastic Materials Inc. Farmingdale, USA NY) at Duke University in a 30° C environmental chamber. Widows were each fed a cricket weekly when not being used for experiments. The experimental microhabitat chambers, hereafter referred to as ‘widow-towers’, consisted of 26 x 30 x 50 cm glass reptile terrariums flipped so that the long side was oriented vertically, and the wire mesh that usually makes up the top of the terrarium was oriented vertically as well (Figure 17 in Appendix A). In each widow-tower, three false retreats consisting of black spray-painted (The Valspar Corporation, Chicago, IL, USA) 10 x 10 x 10 cm boxes (AMAC Plastic Materials Inc. Farmingdale NY, USA) were placed along one side at the bottom, midpoint, and top of each widow tower. Each box had a 2.5 cm wide wooden plank extend from the box to the wire mesh. This allowed the widows access to all the retreats (Figure 17 in Appendix A).

Each widow-tower was disassembled and washed thoroughly with a bleach water solution prior to each trial to remove any possible silk cues (e.g. Johnson, Revis et al. 2011). At the beginning of an experimental session, one widow was placed at the bottom of a widow tower. The widow’s three-dimensional position was recorded 72

hours after placement, a time that has shown final settlement in other species within the *Latrodectus* genus (Johnson, Revis et al. 2011). Because clumping of widows near retreats was expected and therefore the data would not be normally distributed, the height of each widow was scored as low (0-16 cm), middle (17-33 cm), or high (34-50 cm).

Microhabitat choice data were then analyzed using a chi-square goodness of fit test in R.

2.5.2 Results

The dorsally all-black *L. mactans* (n=20) were more likely to settle lower in the widow-towers than the dorsally red spotted *L. variolus* (Figure 8; n=27; chi-squared goodness of fit test, $p < 0.05$). This is consistent with field observations that suggest *L. mactans* is found lower to the ground than *L. variolus* in sympatric habitats (Bhatnagar and Rempel 1962, Kaston 1970).

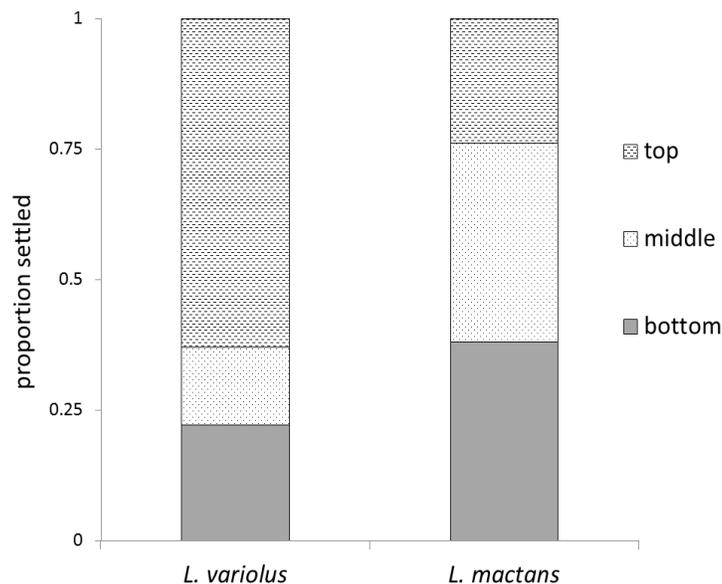


Figure 8: Microhabitat choice data for two species of black widows that can be found in the same habitats. After 72 hours, the dorsally colored *L. variolus* (n=27) settled in microhabitats that were higher off of the ground than *L. mactans* (n=20; $p < 0.05$; chi-squared goodness of fit test).

2.6 Discussion

The results from these experiments are consistent with the hypothesis that North American black widow coloration has evolved to deter avian predators while being inconspicuous to insect prey. We found that if a wild bird sees a black widow model, they are nearly three times more likely to attack an all-black model than one with an hourglass (Figure 3). While previous studies have shown that birds generally avoid predominantly red insects with some black coloration (Jones 1932), our study suggests that wild birds also avoid predominantly black spiders with some red coloration.

Although black widows are primarily nocturnal and the birds studied are primarily diurnal, there are ample interactions between black widows and birds that

could warrant a predator-deterring signal. First, black widows and birds may interact during dawn and dusk when both are active. Additionally, if a black widow is not fully hidden by their retreat, or if the retreat is disturbed, the black widow would be visible during diurnal hours. Lastly, black widows are generalist predators, and in some cases their diet includes many species of insects that are primarily active during the day (Hódar and Sánchez-Piñero 2002, Salomon 2011). For example in British Columbia, >80% of items in the diet of *L. hesperus* consisted of beetles, most of which were from three primarily diurnal families (Salomon 2011). Therefore birds may view black widows when the black widow emerges to subdue their diurnal prey. It is important to note that although a few animals are capable of seeing color during nocturnal illumination levels (Kelber, Balkenius et al. 2002, Roth and Kelber 2004), color vision may be rare in primarily nocturnal animals, suggesting that the coloration of black widows may have limited functionality at night (although it is possible there could be some achromatic information present). Thus rare events during diurnal hours may play the largest role in selecting coloration on a primarily nocturnal animal.

We also found evidence that suggests that black widow coloration may be aposematic (*i.e.* some birds benefit from not attacking black widows) and does not deter birds through a different mechanism [such as neophobia (fear of new prey objects) or Batesian mimicry (resembling dangerous individuals)]. Birds with the shortest beaks were least likely to attack either model in our study (Figure 4). Because the defenses of

the black widow (web and venom) are only effective at short ranges, black widows are most dangerous to birds with short beaks. Bird species with longer beaks, such as the Hoopoe (*Upupa epops*) in Spain, can attack black widows with few negative consequences and have been reported as a common predator (see Hódar and Sánchez-Piñero 2002). If black widow coloration is aposematic, we would expect it to only deter short-beaked birds because long-beaked birds would still benefit from attacking a black widow. However, if black widow coloration deters birds through neophobia or Batesian mimicry, we would expect no correlation between beak size and tendency to attack.

The red-and-black coloration used by black widows has a Euclidean color distance that is ~2 times larger for a typical bird than a typical insect (Figure 6). In addition, it presents the longest wavelength photoreceptor of a typical bird ($\lambda_{\max} = 606$ nm) with a Weber contrast that is ~3.5 times greater than that of a typical insect's longest wavelength photoreceptor ($\lambda_{\max} = 520$ nm). Although an insect's green-sensitive photoreceptor can still detect red light, the color distance between the red and black regions of a black widow may be small compared to other differences in contrast in a visual scene. Of color combinations that are easily visible to birds, the red-and-black color combination used by black widows is likely one of the least visible to most insects. For example, if the coloration consisted of white-and-black or yellow-and-black rather than red-and-black, insects would perceive both a greater color distance and a greater long-wavelength photoreceptor contrast between black widow color components.

Insects could then use this information to avoid the web of the black widow or escape from a black widow attack, thus lowering the black widow's foraging ability.

Evidence from a comparative approach also suggests that black widow coloration is under selection to be conspicuous to predators but inconspicuous to prey. An ancestral reconstruction shows that the common ancestor of all black widows likely had dorsal red coloration (Figure 7). At some point during their evolutionary history, two species of the North American black widows (*L. hesperus* and *L. mactans*) lost all red dorsal coloration [although rarely adult females of the species can be found with red dorsal spots (Kaston 1970)]. In contrast, another North American species (*L. variolus*) typically has red dorsal coloration (Figure 1), although it is not clear if its dorsal coloration was retained throughout the entire lineage (Figure 7). We found that the differences in dorsal coloration between *L. mactans* and *L. variolus* were accompanied by a difference in microhabitat choice. In laboratory experiments, *L. variolus* settled farther above ground level in the three dimensional habitats than *L. mactans* (Figure 8), consistent with earlier field observations by Kaston (1970). The difference in coloration between the species follows what would be expected if both predator and prey visual systems were simultaneously shaping black widow coloration. *L. mactans* prefers microhabitats that are relatively close to ground level. Therefore *L. mactans* has a low probability that an avian predator views their dorsal side; however, insect prey are still likely to view the dorsal side of *L. mactans* because black widow webs are designed to

catch prey that approach from below (Blackledge and Zevenbergen 2007). In *L. mactans*, a lack of any red dorsal coloration suggests the benefits of a downward facing aposematic signal are outweighed by the costs of potential prey using that signal to avoid the black widow. Alternatively, *L. variolus* is found in microhabitats that are farther above ground level. In these locations predators can approach from any direction, including from below. Therefore *L. variolus* has a higher probability that a predator approaches them from below than *L. mactans*, and as a result the benefits of a downward facing aposematic signal may outweigh the costs of potential prey using the signal to avoid the black widow. Previously, differences in aposematic coloration between species have been explained by differences in toxicity (Mallet and Joron 1999, Wang and Shaffer 2008, Wang 2011), or differences in predators (Endler and Mappes 2004, Chouteau and Angers 2011). However in the case of black widows, neither seems likely. At least in humans, bites from all species of black widow are treated with the same clinical methods (Clark, Wethernkestner et al. 1992), and no species of North American black widow is considered to be more dangerous than the others. In addition, because *L. mactans* and *L. variolus* are often sympatric (Kaston 1970), differences in predators between the species may be minimal.

Researchers have often discussed the 'cost of conspicuousness' of an aposematic signal as an initial cost imposed by naïve predators (Mallet and Singer 1987, Ruxton, Sherratt et al. 2004, Marples, Kelly et al. 2005). However, even when an aposematic

signal is established within a population a conspicuous signal may lower the foraging rate of the signaler, as potential prey may use the signal to avoid the individual (Grether and Grey 1996, Kats and Dill 1998, Ortolani 1999, Baird 2008). Similarly, the defense of an aposematic animal may not function against certain parasites, predators, or rivals (e.g. Eisner, Baldwin et al. 1993, Eisner, Eisner et al. 1998, Eisner and Eisner 2000), and being conspicuous to these individuals may lower the fitness of the signaler.

We suggest that the black-and-long-wavelength (*i.e.* yellow, orange and red) color patterns commonly associated with aposematic signals may not be solely driven by interactions with the signal's intended receiver (reviewed in Stevens and Ruxton 2012), but also by reducing the costs of conspicuousness to many potential eavesdroppers that lack refined red vision (such as insects or most non-primate mammals). The frequency of these colorations has usually been explained by a variety of receiver effects; in particular environments and with particular receivers, black-and-long-wavelength coloration may be a more effective aposematic signal than other possible color combinations (Stevens and Ruxton 2012). However, the American black widows illustrate another advantage of red-and-black aposematic signals -- being inconspicuous to prey. In any situation where the proposed receiver of an aposematic signal is a bird or human, and the signaler is also a predator of other animals, a red-and-black color scheme may be the ideal coloration to balance the competing selective pressures of conspicuousness to predators and inconspicuousness to prey. Even if just a few aposematic species benefit from the

potential eavesdropper avoidance effect of red-and-black coloration, these species may drive the evolution of coloration in other aposematic species towards long wavelength coloration through Müllerian mimicry and receiver generalization (Benson 1972, Plowright and Owen 1980, Gamberale-Stille and Tullberg 1999, Mallet 1999, Speed 2000, Svadova, Exnerova et al. 2009). Because eavesdroppers (such as prey) may impose a consistent cost of conspicuousness to aposematic individuals, those aposematic signals that reduce the effects of eavesdroppers (while maintaining their effectiveness towards intended receivers) may be more likely to both initially evolve and persist through evolutionary time. Future studies on the evolution of aposematic signals should examine how directional selection on aposematic coloration by animals other than the intended receiver influence mimicry and the diversity of aposematic patterns seen in nature.

3. Variation in hourglass morphology among nine sites of North American black widows (genus *Latrodectus*)

3.1 Introduction

Many predators learn to avoid unpalatable or otherwise dangerous prey because the prey displays certain consistent color patterns known as aposematic coloration (Poulton 1890, Cott 1940, Ruxton, Sherratt et al. 2004). However some potential predators still make mistakes and attack aposematic individuals. If a predator has an unpleasant experience with one aposematic individual, individuals with similar signals may be more likely to be avoided by the predator in the future because of learning and template formation (Benson 1972, Roper and Cook 1989, Yachi and Higashi 1998, Mallet 1999). As a result of predator cognition, classical aposematic theory predicts that warning signals should vary little within a location (Joron and Mallet 1998, Kapan 2001, Rowland, Ihalainen et al. 2007). Notably, this prediction applies both to members of the same species and to members of different species that are well-defended (Mullerian mimicry).

However, the assumption that natural selection should limit variation in aposematic signals both within and between species has been challenged by several studies. Aposematic signals may vary between individuals due to differences in development or diet (Darst, Cummings et al. 2006, Lindstedt, Eager et al. 2011), as well as between populations due to different ecological conditions, such as predator type and abundance (Endler and Greenwood 1988, Mallet and Joron 1999, Endler and Mappes 2004). Aposematic signals may also vary because of the cognition and sensory

capabilities of the potential predator. Differences in aposematic coloration and patterning may be selectively neutral if predators view aposematic signals categorically (rather than continuously), and the aposematic signal parameters fall within the predator's perceptual bounds; *i.e.* signals with some variation may all have the same effect on predators (Holloway, Gilbert et al. 2002, Lindström, Alatalo et al. 2004, Siddiqi, Cronin et al. 2004, Puurtinen and Kaitala 2006). Altogether, recent work suggests that variation in aposematic signal form may actually be common in nature (Stevens and Ruxton 2012).

Adult female black widow spiders (genus *Latrodectus*) are a useful model system to study variation in aposematic signals because they allow us to examine how mimicry, species type, and condition shape the form of an aposematic signal. Previous work on this genus has suggested that the red-and-black coloration of black widows (specifically the hourglass) functions as an aposematic signal to birds (Brandley et al., in review); however, other predators may be relevant as well (D'Amour, Becker et al. 1936). Within *Latrodectus*, hourglass shape does not follow the expectations of classical aposematic theory because it is quite variable; in some individuals, the hourglass is barely visible to human observers, while in other individuals it covers most of the ventral abdomen (Levi 1959, Kaston 1970). This variation appears to be continuous, which is in contrast to many other aposematic signals where variation appears to be discrete and controlled by a genetic polymorphism (e.g. Mallet and Joron 1999, Siddiqi, Cronin et al. 2004, Noonan and Comeault 2009). In addition, three different species of black widows are found in the United States (Garb, Gonzalez et al. 2004), allowing us to examine variation both

between and within species. Lastly, aposematic signal form may vary with both immediate and long term condition of the signaler. Black widow size measurements can simultaneously serve as proxies for both types of condition. In spiders, immediate condition is often represented by abdomen size and/or weight relative to body size, because both measurements increase rapidly with feeding (Anderson 1974, Andrade 1998, Pasquet, Leborgne et al. 1999). Alternatively, cephalothorax and leg length reflect long term condition because they do not change with one feeding but instead represent development and growth over time (e.g. Elias, Mason et al. 2003, Elias, Lee et al. 2006).

Previous descriptions of variation in hourglass shape between and among the three North American species [*L. hesperus*, *L. mactans*, *L. variolus*; (Kaston 1970)] have been qualitative, and hourglass shape variation has not yet been quantified over a large region, nor have the causes of hourglass variation been investigated. Here we address these shortcomings by quantifying hourglass shape variation across nine black widow sites using morphological markers. We then examine to what levels mimicry, species, and condition predict the variation in hourglass shape.

3.2 Methods

3.2.1 Study animals

Black widows were obtained from nine different sites across the United States via private collectors (Table 8 in Appendix C). Only wild-caught adult female black widows were used. Widows were identified at the species level by a combination of dorsal coloration pattern (not hourglass pattern, which is ventral) and geographical location. Specimens of *L. hesperus* were obtained from Placer County, CA (n = 12), Ventura

County, CA (n = 19), Bernalillo County, NM (n = 16), and El Paso County, TX (n = 20). *L. mactans* were obtained from Chaves County, NM (n = 20), Clarke County, AL (n = 19), and Citrus County, FL (n = 9). *L. variolus* were obtained from Camden County, MO (n = 8), and Jefferson County, KY (n = 10). One *L. hesperus* from Placer County, CA had no visible hourglass and was excluded from all analyses. Black widows were housed individually in clear plastic containers (10 x 10 x 10 cm; AMAC Plastic Materials Inc. Farmingdale, NY, USA) at Duke University in a 30° C environmental chamber for at least two weeks prior to experimentation. Each widow was fed one cricket approximately one week prior to experimentation.

In addition to identifying individuals to species, we also classified each collection site into one of three broader geographical regions (western, southwestern, eastern) so that we could examine the potential for Müllerian mimicry (Müller 1879) over larger regions. The western region (n = 30) consisted of the two sites in California from Placer and Ventura County, the southwest region (n = 56) consisted of Bernalillo County, NM, El Paso County, TX, and Chaves County, NM, and the eastern region (n = 46) consisted of the four other sites (Camden County, MO; Clarke County, AL; Jefferson County, KY; Citrus County, FL).

3.2.2 Size measurements and photography

At the time of analysis, each widow was anesthetized with nitrogen gas (~10 minutes) until they remained motionless. We then recorded each widow's weight, cephalothorax length, maximum cephalothorax width, and the average leg length. Following Andrade's (1998) protocol in redback spiders, leg length was measured as the

total length of the patella and tibia of the first legs of each individual. Each widow was photographed individually using a Nikon D-5200 camera with an AF-S DX Nikkor 18-55 mm f/3.5-5.6G VR lens (Nikon Corporation, Japan). The widow was positioned on its dorsal side so that the ventral hourglass was displayed to the camera and remained flat relative to the plane of the picture. A ruler placed at the same height as the hourglass was used as a scale bar in each picture. Multiple photos of each widows were taken, and the one with the clearest hourglass was chosen for further analysis in ImageJ (Schneider, Rasband et al. 2012).

3.2.3 Hourglass shape modeling

Hourglass shape was modeled by approximating the hourglass of each black widow as an upper and lower trapezoid that may or may not overlap (Figure 18 in Appendix C). In ImageJ markers were placed at eight positions along the hourglass corresponding to the corners of both the upper and lower trapezoid. If the trapezoids overlapped to form one continuous hourglass, the narrowest section of the hourglass was used to mark both the bottom of the upper trapezoid and the top of the lower trapezoid. x and y scores for each marker were then gathered in ImageJ. Because the angle at which the hourglass was viewed differed between photographs, all points were then rotated via trigonometry so that the sum of the slope of the top of the upper trapezoid and the slope of the bottom of the lower trapezoid were equal to 0 (yellow lines in Appendix C). Points were then normalized around the centroid (green point) of all eight markers using known measurements based on the size standard in each picture. Thus, for each black widow, we had eight points with x and y values of known distances from the centroid. Because of the

high degree of correlation between points caused by bilateral symmetry, we averaged the absolute x and y distances of analogous points across a vertical line of symmetry (white line). This gave us four x values and four y values that were not as correlated, and thus could be used in further analysis without violating any statistical assumptions regarding independence.

3.2.4 Principal Component Analysis (PCA) of hourglass shape (hgPCA) and black widow size (sPCA)

We performed a PCA (correlation matrix type) on the hourglass markers of all 132 black widows in JMP Pro (SAS Institute Inc. 2013). We also performed a PCA (correlation matrix type) on 130 black widows for which we had complete size data for all four parameters (see *Size measurements and photography*). Two *L. hesperus* from Ventura County, CA were excluded from this analysis because of incomplete size measurements. For both PCAs we kept any principal components (PCs) that explained more than 10% of the variation for use in further analyses.

3.2.5 Discriminate Function Analysis for species, geographical region, and site

Because each collection site represented only one species from one geographical region, we were unable to simultaneously control for species, geographical region, and site; instead we performed three different linear discriminate function analyses (DFA) separately examining categorizations to species, geographical region, and site by hourglass shape. DFAs were performed in R (R Core Team 2013) using the `lda` function of the MASS package (Venables, Ripley et al. 2002). All values reported use the

all-but-one (jackknife) approach that separately predicts each individual classification by using all other individuals. The DFA by species showed a strong bimodal distribution for *L. variolus* with each *L. variolus* site falling in its own cluster. Therefore we categorized each *L. variolus* site as a separate species for the purposes of the species DFA. This categorization changed the result of the species DFA very little, improving its accuracy by less than 3%, while eliminating any violations of normally distributed data that the DFA requires.

For the DFA by site, we also examined all misclassified individuals (n = 75) to determine if they were misclassified into a site of the correct species and/or correct geographical region. We then compared these values to those expected by random chance via a Fisher's exact test.

3.2.6 Generalized Linear Models of hourglass shape

We ran six linear regressions in R (R Core Team 2013) using the GLM function of the lme4 package (Bates, Maechler et al. 2014). Each regression looked at how one of the three hourglass shape principal components ('hourglass size', 'separation between parts', 'slenderness'; see *Principal Component Analysis of hourglass shape (hgPCA) and black widow size (sPCA)* results below) could be explained by either species or site location and the three black widow size principal components ('overall size', 'relative leg size', 'immediate condition'). Each generalized linear model was first fit using the Bayesian Information Criteria (Schwarz 1978) and then the final p values for each independent variable within each model were adjusted for multiple samplings via Bonferroni corrections (n=6).

3.3 Results

3.3.1 Principal Component Analysis of hourglass shape (hgPCA) and black widow size (sPCA)

We performed two separate principal component analyses on hourglass shape and black widow size, and so we refer to all principal components as either hgPC for the hourglass shape PCs or sPC for the black widow size PCs. Each of the first three hgPCs explained at least 10% of the variation in hourglass shape and collectively explained 84.6% of the total variation (Table 1; all eigenvalues > 1.0, Bartlett's test $p < 0.001$); hgPC1 explained 50.2% of the total variation and had loadings that made the hourglass larger in all directions. As such we termed hgPC1 'hourglass size'. hgPC2 explained 21.8% of the total variation and had large loadings for increased separation of the top and bottom trapezoids. As such we termed hgPC2 'separation between parts', with higher scores representing a larger separation between hourglass parts. hgPC3 was termed 'slenderness' because its loadings represented differences in hourglass slenderness, with a higher score representing a more slender, narrower hourglass; it explained 12.6% of the variation.

For black widow size, each of the first three sPCs again explained at least 10% of the variation in black widow size and collectively explained 94.6% of the total variation (Table 2; all eigenvalues > 0.5, Bartlett's test $p < 0.0001$). sPC1 explained 57.2% of the variation in black widow size and was termed 'overall size' because its loadings were substantially positive for all four size measurements. sPC2 explained 21.4% of the variation in black widow size and was termed 'relative leg size' because its loading for

leg size was strongly positive while all other loadings were near zero. sPC3 explained 15.0% of the variation and was termed ‘immediate condition’ (e.g. Andrade 1998, Elias, Lee et al. 2006) because its loadings represented an increase in weight with a slight decrease in all other size measurements.

Table 1: Hourglass shape principal components and loading scores

	PC1	PC2	PC3
general description	"hourglass size"	"separation between parts"	"slenderness"
percent variation	50.1	21.8	12.6
eigen value	4.0	1.7	1.0
visualization			
PC loading scores			
upper trapezoid top height	0.65	0.44	0.47
upper trapezoid top width	0.89	0.11	0.02
upper trapezoid bottom height	-0.48	0.76	-0.01
upper trapezoid bottom width	0.79	0.09	-0.45
lower trapezoid top height	-0.46	0.86	0.02
lower trapezoid top width	0.61	0.45	-0.52
lower trapezoid bottom height	0.73	0.11	-0.06
lower trapezoid bottom width	0.91	-0.04	0.56
	PC1	PC2	PC3
our description	"overall size"	"relative leg size"	"immediate condition"
percent variation	57.2	21.4	15

Table 2: Black widow size principal components and loading scores. ¹: Similarly to Andrade (1998), leg length was measured as the combined length of the patella and tibia of the first leg.

	PC1	PC2	PC3
our description	"overall size"	"relative leg size"	"immediate condition"
percent variation	57.2	21.4	15
eigen value	2.3	0.9	0.6
PC loading scores			
weight	0.74	0.07	0.67
cephalothorax length	0.85	-0.35	-0.20
cephalothorax width	0.87	-0.21	-0.25
leg length ¹	0.52	0.83	-0.20

3.3.2 Discriminate Function Analysis via species, geographical region, and site

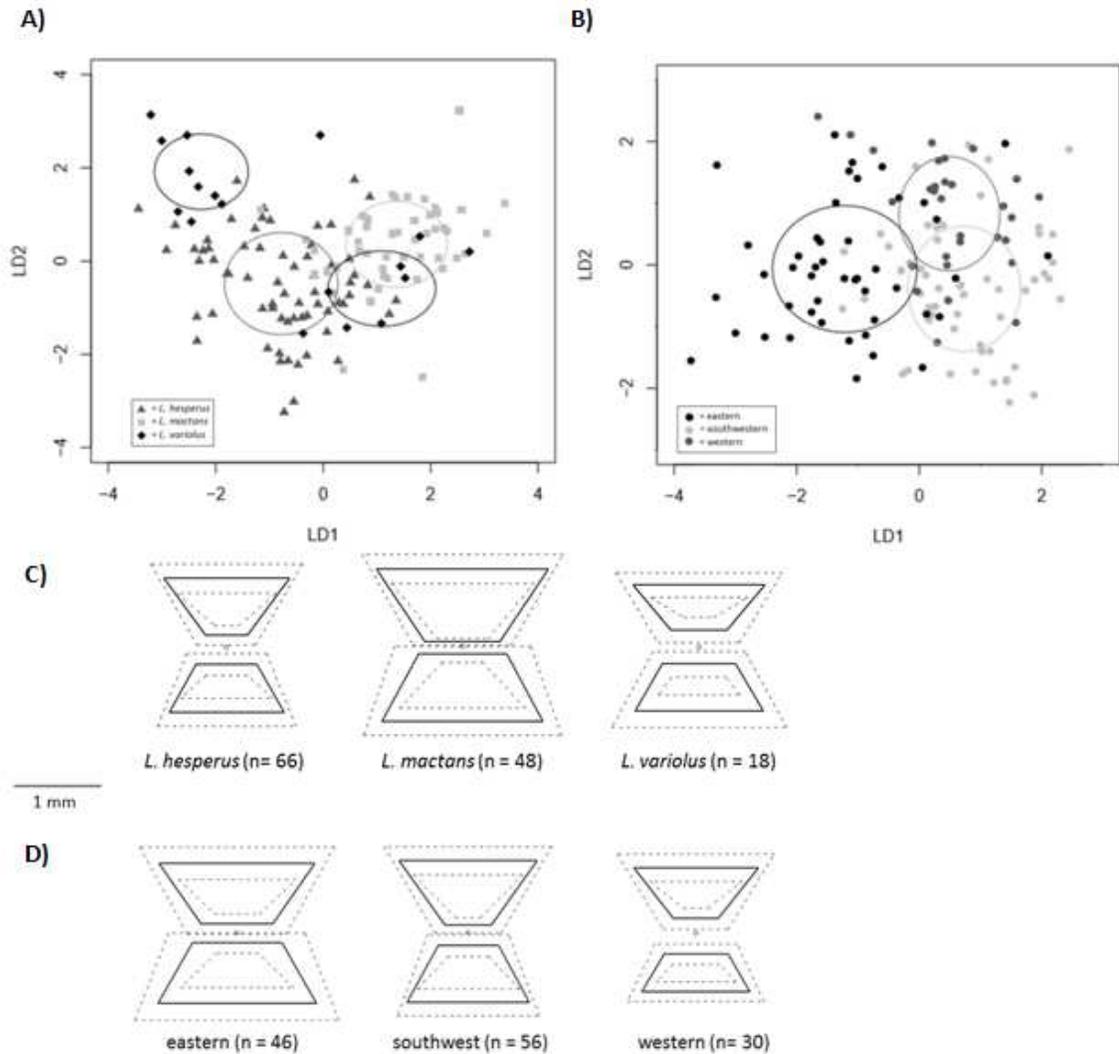


Figure 9: A-B) Linear discriminant function analysis (DFA) of hourglass shape by A) species and B) geographical region. A) The DFA correctly assigns 75% of individual black widows to the correct species. Note that because *L. variolus* shows a bimodal distribution of hourglass shape, each population of *L. variolus* (black diamonds) was considered to be a separate species for the analysis. B) The DFA correctly assigns 67% of individual black widows to the correct geographical region. C-D) Mean hourglass shape by C) species and D) geographical location. Dashed lines represent hourglasses that were one standard deviation larger or smaller than the mean in all parameters.

When controlling for black widow size PCAs, *L. hesperus* differed from *L. mactans* in many traits measured ($p < 0.05$), with *L. variolus* often falling intermediately.

Using only hourglass shape data, both the DFA by species and by geographical region showed improved accuracy over that of chance (Figure 9); categorizations by species performed only slightly better (75% accurate; expected accuracy by chance = 39%) than those by geographical region (67% accurate; expected accuracy by chance = 35%). The DFA by site correctly predicted site identity for 43% of black widows (expected accuracy by chance = 12%). Individuals that were assigned to an incorrect site were assigned to a site of the same species, as opposed to a site of a different species, 62% more often than expected by chance (Fisher's exact test; $p < 0.05$). No significant trend was found in incorrect assignment by geographical group (24% less often than expected by chance; Fisher's exact test; $p = 0.44$).

3.3.3 Generalized Linear Models (GLM) of hourglass shape

The most parsimonious GLMs suggest that hourglass shape can be explained by black widow size (sPC1) and immediate condition (sPC3), even when controlling for significant differences in species and/or site differences (Table 3). When running the GLMs with species variables, both the size of the hourglass (hgPC1) and the separation between hourglass parts (hgPC2) can be explained by a combination of species identity, black widow overall size (sPC1), and immediate condition (sPC3). *L. mactans* was found to have both a significantly larger hourglass size ($p < 0.001$) and a smaller separation between hourglass parts than *L. hesperus* ($p < 0.05$), while *L. variolus* showed no significant differences compared to both other species. Overall larger (sPC1) black

widows showed increased hourglass size (hgPC1; $p < 0.001$) and a larger separation between hourglass parts (hgPC2, $p < 0.001$) as did those with an increased immediate condition (sPC3; $p < 0.001$, $p < 0.05$ respectively). The most parsimonious GLM suggested that the slenderness (hgPC3) of the hourglass could be explained by a combination of species and immediate condition (sPC3). Both *L. mactans* ($p < 0.001$) and *L. variolus* ($p < 0.001$) had wider hourglasses than *L. hesperus*, and black widows with increased immediate condition (sPC3) showed more slender hourglasses ($p < 0.01$). Relative leg size (sPC2) had no significant effect on any hourglass shape parameter.

Table 3: Generalize Linear Models (GLM) results. Check mark indicates a significant difference between species or sites. + indicates a significant positive affect.

		GLM with species predictors				
		species	bw size (PC1)	leg size (PC2)	immediate condition (PC3)	significant interactions
response	hg size (PC1)	✓				+
	separation between parts (PC2)	✓				+
	slenderness (PC3)	✓				+
		GLM with site predictors				
		site	bw size (PC1)	leg size (PC2)	immediate condition (PC3)	significant interactions
response	hg size (PC1)	✓				site:size
	separation between parts (PC2)	✓		+		+
	slenderness (PC3)	✓		+		+

When running the GLMs by site rather than species (Table 3), the most parsimonious models suggest that hourglass size (hgPC1) can be explained by site ($p < 0.001$) and the interactions between site and size ($p < 0.001$). Both the separation between hourglass parts (hgPC2) and the slenderness of the hourglass (hgPC3) were significantly affected by site, black widow overall size (sPC1), and immediate condition (sPC3). Larger black widows had a larger separation between hourglass parts ($p < 0.001$) and a more slender hourglass ($p < 0.001$), and black widows in better immediate condition

showed the same pattern ($p < 0.001$ for both hgPCs). Relative leg size (sPC2) had no significant effect on any hourglass shape parameter.

3.4 Discussion

3.4.1 Validity of the hourglass model technique

Our quantitative model agrees with previous qualitative descriptions of black widow hourglass shape (Levi 1959, Kaston 1970): the principal components analysis of hourglass shape based on our morphological markers technique (Table 1) suggests that around half of the variation in hourglass shape is in hourglass size with another combined 34% of the variation occurring as separation between upper and lower hourglass parts and the slenderness of the hourglass. While our approach may have lost some of the micro-variation in hourglass shape (such as a rounding of hourglass corners or unevenness in lines) many of these differences could probably not be resolved by potential predators at a useful distance because of limits of spatial acuity (Harmening, Nikolay et al. 2009, Land and Nilsson 2012). In addition, each part of the hourglass varied in ways that agreed with the shapes used previously to describe the hourglasses such as ‘triangular’ and ‘rectangular’, and ‘trapezoidal’. We believe that the cost of losing some of the micro-variation in hourglass shape was outweighed by the advantages of being able to quantify hourglass shape and its variation.

3.4.2 Variation within sites and between sites of the same species

The variation in hourglass shape both within sites and between sites (Figure 10) appears to be inconsistent with the expectations of classical aposematic theory (Müller

1879, Joron and Mallet 1998, Kapan 2001, Rowland, Ihalainen et al. 2007). A linear discriminant function analysis (DFA) was only able to correctly assign 43% of the black widows studied to their correct site (Figure 11), which is an improvement over random chance (12%) but does not represent perfect mimicry of hourglass shape. Note that we do not know the relatedness between individuals at a given site; if individuals in our study were related we may have underestimated hourglass shape variation and overestimated the accuracy of our site DFA between unrelated individuals.

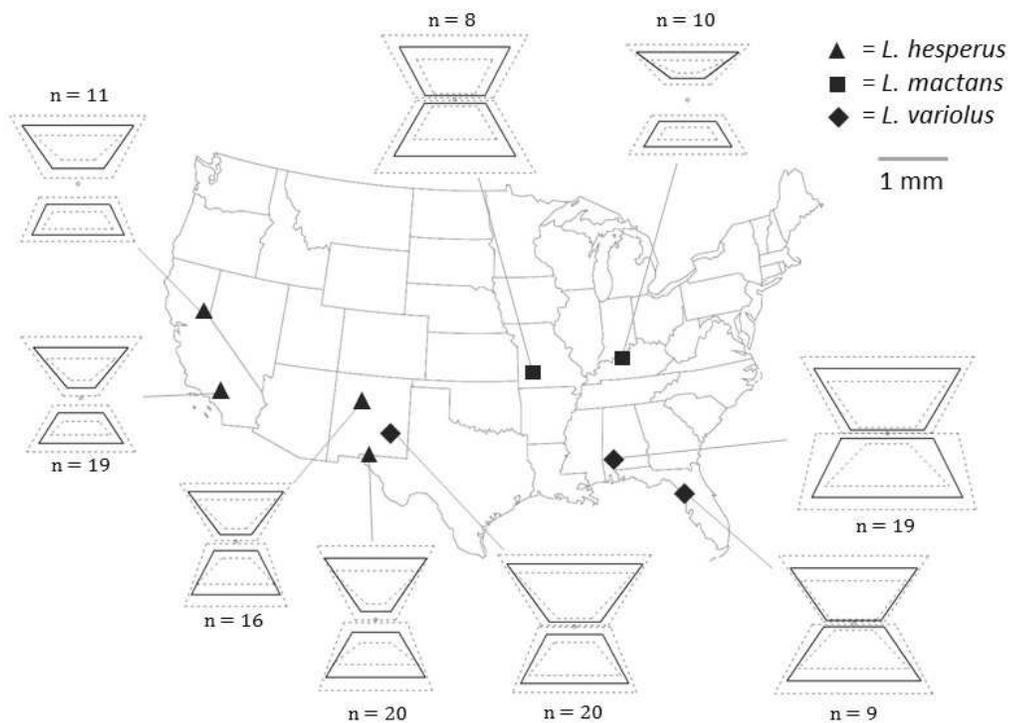


Figure 10: Mean hourglass shape across nine sites of North American black widows (genus *Latrodectus*). Dashed lines represent hourglasses that were one standard deviation larger or smaller than the mean in all parameters. Significant differences in hourglass shape were found both between sites and between species.

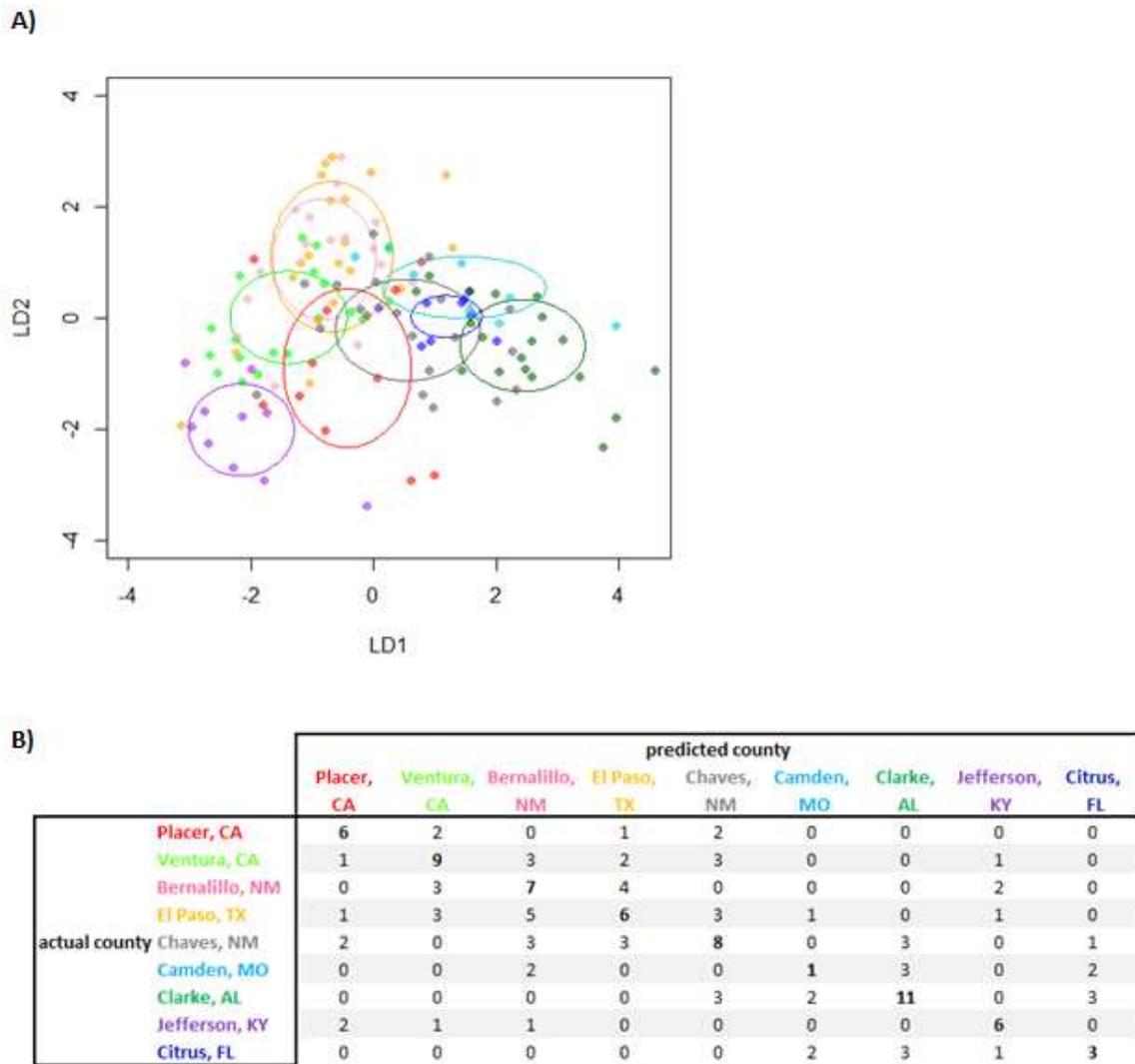


Figure 11: Linear discriminant function analysis (DFA) of hourglass shape by site. A) The DFA correctly assigns 43% of individual black widows to the correct site. See B for the color key of each site. B) Assignment of individual black widows by site.

Numbers in bold along the diagonal indicate assignment to the correct site. Individuals that were assigned to an incorrect site were assigned to a site of the same species more often than expected by chance (Fisher's exact test; $p < 0.05$), while no significant trend was found in incorrect assignment by geographical region (Fisher's exact test; $p = 0.44$).

While some of the variation within a species (both between and within sites) can be attributed to differences in black widow size (see below), hourglass shape still varied

significantly, even when controlling for black widow size (Table 3). This contradicts Müller's theoretical assumption that uniformity should be favored because it expedites predator learning and recognition of an aposematic display (Müller 1879, Sherratt 2008). One possibility is that sexual selection may have caused variation in aposematic signal form (Siddiqi, Cronin et al. 2004, Maan and Cummings 2008, Maan and Cummings 2009, Stevens and Ruxton 2012). Black widow spiders are a sexually dimorphic species (Levi 1959, Kaston 1970), and it is known that some species of male widows prefer fed, virgin females (MacLeod and Andrade 2014, Baruffaldi and Andrade 2015). However, while black widow vision is unstudied, their eyes are relatively small (diameter of ~0.2 mm), which would limit spatial acuity and their ability to resolve fine scale hourglass differences (Johnsen 2012, Land and Nilsson 2012). Additionally, current research suggests that mate discrimination by male widows is based on olfaction and tactile information (Kasumovic and Andrade 2004, Johnson, Trubl et al. 2011, MacLeod and Andrade 2014, Baruffaldi and Andrade 2015) rather than vision. Alternatively, natural selection could act to decrease variation in hourglass displays until a threshold of similarity is reached beyond which random drift is permitted. Beyond this threshold the predator either lacks the refined spatial vision to recognize variable hourglass shapes as different (Marshall 2000; Caves et al. in review, Baldwin and Johnsen 2011), or the predator does not selectively discriminate between different hourglass shapes (Holloway, Gilbert et al. 2002, Rowe, Lindström et al. 2004, Amézquita, Castro et al. 2013). Studies of other aposematic organisms have shown that some avian predators innately avoid most conspicuous displays, which thus allows for more individual variation in coloration

(Smith 1975, Caldwell and Rubinoff 1983, Siddiqi, Cronin et al. 2004). Notably for black widows, naïve avian predators may permit more variation than experienced ones (Rowe, Lindström et al. 2004), which may help increase the variation in hourglass shape within a site because the aposematic signal in black widows may be maintained by relatively rare interactions (see Brandley et al., in review).

3.4.3 The effects of species and geographical region

The distribution of our data (each site is just one species and in just one geographical region) makes it difficult to independently separate the effects of site, species, and geographical region on hourglass shape. This is especially true when trying to separate the effects of species from those of geographical region: each geographical region is biased towards one species (the western region was all *L. hesperus*, the southwest region was 64% *L. hesperus*, and the eastern region was 60% *L. mactans*). However, the DFA by site suggests that differences in species --- and not regionalized mimicry ---- lead to improved accuracy of both the DFA by species (75% accurate; Figure 9) and by geographical region (67% accurate; Figure 9). Those individuals that were incorrectly assigned by the site DFA (Figure 11) were assigned to a site of the same species more often than expected by chance (Fisher's exact test; $p < 0.05$), while no significant trend was found in incorrect assignment by geographical group (Fisher's exact test; $p = 0.44$). Therefore it appears that differences in hourglass shape between larger regions may actually reflect differences in species composition rather than any selection for mimicry.

Our quantitative models of hourglass shape between species (Figure 9c; Table 3) agree with previous qualitative descriptions (Kaston 1970): compared to *L. mactans*, *L. hesperus* has a smaller, more slender hourglass with an increased distance between the upper and lower hourglass parts (Figure 9c). The two populations of *L. variolus* examined showed a bimodal distribution with one population resembling *L. hesperus* and the other resembling *L. mactans* (Figure 9a). The ultimate mechanisms behind these species differences are unclear. One hypothesis is that the hourglasses are not only aposematic signals but also function as species-specific mating signals. However, like sexual selection within these species (see previous section), visual cues may not be needed because of cues in other modalities. In addition, the bimodal distribution of *L. variolus* hourglass shape makes this hypothesis unlikely. Another hypothesis is that differences in hourglass shape could reflect different ecological and or dietary conditions (Mallet and Joron 1999, Endler and Mappes 2004, Darst, Cummings et al. 2006, Lindstedt, Eager et al. 2011). These differences would have to occur on a relatively narrow scale (such as in microhabitat), as we previously saw that regional differences in hourglass shape are better explained by species than region. Lastly, the differences in hourglass shape between species could be non-adaptive and reflect drift (Goodman, Kelley et al. 2015) or be genetically linked to other traits.

3.4.4 The effects of size on black widow hourglass shape

While the DFAs are useful because they show all the variation potential predators would encounter within each site (Figure 11), species (Figure 9c), or geographical region (Figure 9d), they do not account for differences in black widow size (Table 2) that also

influence hourglass shape. Our size PCA suggested that most of the variation in black widow size occurred in overall size, relative leg length, and immediate condition (Table 2). The most parsimonious generalized linear models (GLM) suggest that both site and black widow size influence hourglass shape when running the GLMs with site information, and that both species and black widow size influence hourglass shape when running the GLMs with species information (Table 3). Specifically, we found that overall black widow size (sPC1) and immediate condition (sPC3) often had positive effects on hourglass shape PC scores, while relative leg size (sPC2) had no significant effect on any hourglass shape trait (Table 3). Because larger widows in better immediate condition may be more likely to have better defenses [including larger fangs and increased web secretions (Vetter 1980)], the observed trends in hourglass shape could make it possible for predators to use hourglass shape as an honest indicator of potential danger (Zahavi 1975, Zahavi 1977, Smith and Harper 1995). The exact mechanism that would ensure honesty is unknown; however, it seems likely that both increased size and increased immediate condition would physically stretch the hourglass, making it possible to function as an index signal. It remains to be seen if predators use differences in hourglass shape to determine whether they will attack and if differences in hourglass shape correlate with increased danger to predators.

3.5 Conclusion

This study adds to a growing body of literature which suggests that mimicry in nature is not as perfect as classical aposematic theory suggests (Mallet and Joron 1999, Lindström, Alatalo et al. 2004, Siddiqi, Cronin et al. 2004, Darst, Cummings et al. 2006,

Perrard, Arca et al. 2014). We found that black widow hourglass shape varies within and between sites, between species, and with black widow size. Surprisingly we found no evidence for mimicry in hourglass shape across larger regional areas. Future studies should examine factors besides mimicry (such as predator behavior, developmental constraints on hourglass shape, and ecological variables that differ between species/sites) that may help explain the variance in hourglass shape.

4. Eavesdropping on visual secrets

This work has been previously published in the November 2013 issue of *Evolutionary Ecology* (Brandley, Speiser et al. 2013).

4.1 Introduction

Organisms rarely signal in environments that lack potential eavesdroppers. While signalers may benefit if they communicate with intended receivers, they may suffer if their communication is detected by eavesdroppers such as parasites, predators, potential prey or rivals (Cade 1975, Endler 1980, Tuttle and Ryan 1981, Stauffer and Semlitsch 1993, Wagner 1996, Zuk and Kolluru 1998, Peake, Terry et al. 2001, Peake 2005, Kim, Brown et al. 2009, Clark, Roberts et al. 2012). However, because sensory perception often varies both between species (Munz 1958, Lythgoe 1968, Menzel 1979, Marshall, Kent et al. 1999, Briscoe and Chittka 2001, Frentiu, Bernard et al. 2007) and among conspecifics (*e.g.* Jacobs 1984, Gumm, Loew et al. 2012), signalers can lower the costs imposed by potential eavesdroppers—while maintaining the benefits of communication to intended receivers—if they communicate privately with a signal that is less apparent to potential eavesdroppers than intended receivers. Because of the benefits of avoiding detection by potential eavesdroppers, private signals have been suggested in nearly every signaling modality (*e.g.* Williams and Dodson 1972, Hopkins and Heiligenberg 1978, Payne, Langbauer et al. 1986, Narins 1990, Stoddard 1999, Nakano, Skals et al. 2008, Romer, Lang et al. 2010). Notably, much of this work has focused on color signals

(Denton, Gilpinbr et al. 1970, Hinton 1976, Endler 1978, Endler 1980, Endler 1991, Guilford and Harvey 1998, Cummings, Rosenthal et al. 2003, Stevens and Cuthill 2007, Siebeck, Parker et al. 2010, Bybee, Yuan et al. 2012) and polarization signals (Shashar, Rutledge et al. 1996, Chiou, Kleinlogel et al. 2008, Mathger, Shashar et al. 2009, Brady and Cummings 2010). Recently, communication of this sort has gone by a variety of names including “concealed” (Shashar, Rutledge et al. 1996), “covert” (Warrant 2010), “hidden” (Stevens and Cuthill 2007), “private” (Endler 1983), “privileged” (Cronin, Shashar et al. 2003), “secret” (Chiou, Kleinlogel et al. 2008), and “shielded” (Hastad, Victorsson et al. 2005); while the names are different, all of these papers discuss the biological process that we refer to here as private communication.

Most studies on private communication have focused on how private communication benefits a signaler, but private communication channels are almost certainly influenced by the evolution of signalers, intended receivers, and potential eavesdroppers. Examining a potential eavesdropper’s evolution and fitness correlates can (1) help determine the likelihood that private communication channels are stable over evolutionary time, and (2) demonstrate that a signal is adaptively private, not simply less detectable to potential eavesdroppers for non-adaptive reasons. From the perspective of a potential eavesdropper, a private communication channel may pose two different selective pressures. First, such as in species-specific sex pheromone signaling (Lewis and Cane 1990) and some pollination mutualisms (*e.g.* Williams and Dodson

1972, Schaefer, Schaefer et al. 2004, Chen, Song et al. 2009, Svensson, Okamoto et al. 2010), both signalers and potential eavesdroppers may benefit from private communication. Eavesdroppers may benefit in these situations by not responding to irrelevant signals. In these cases, we expect selection on potential eavesdroppers to favor the formation and persistence of private communication channels. In the second case, such as in predator–prey interactions, potential eavesdroppers may be harmed by private communication if not detecting a signal leads to fitness-related consequences such as missed meals (Endler 1980, Cummings, Rosenthal et al. 2003) or increased attacks by signalers (Hinton 1976). Here, we expect selection on potential eavesdroppers to oppose the formation and persistence of private communication channels. Thus, we term private communication detrimental to potential eavesdroppers as protected private communication to emphasize that if a signal is to remain undetectable over time, potential eavesdroppers must face evolutionary barriers that make it unlikely for them to evolve the ability to detect it.

In this review, we examine protected private communication from the perspective of potential eavesdroppers. Throughout, we integrate physiological and behavioral studies on private communication with those on the evolution of sensory systems in potential eavesdroppers. To elucidate our points, we use case studies involving vision, specifically as it relates to the detection of color and polarization signals. Visual signals tend to be displayed more continuously than other types of

signals and are thus more likely to inadvertently reveal a signaler's location (*e.g.* Endler 1980, Lloyd and Wing 1983, Bruce, Herberstein et al. 2001). Signalers can voluntarily discontinue acoustic, electric, or mechanical signals, for example, when eavesdroppers are nearby, but this is less commonly the case for nonbioluminescent visual signals. Thus, we argue that signalers that depend on visual cues are particularly likely to benefit from private communication. Additionally, many researchers have studied the evolution of vision, allowing us to connect what we know about private signals to what we know about the evolution of the visual systems of potential eavesdroppers.

As a first step towards examining how the evolution of potential eavesdroppers may influence private communication channels, we discuss barriers that may allow visual protected private communication to remain private over evolutionary time scales. Although certain signals could remain private over time because of rapidly changing ecological interactions or co-evolutionary arms races, neither of these possibilities are currently supported by published research. Thus, in parts I and II of this paper, respectively, we focus our discussion on the evolutionary constraints and functional costs associated with potential eavesdroppers evolving the ability to detect particular private signals. When considering our findings from parts I and II, as well as evidence that certain potential eavesdroppers have cracked communication channels previously considered private (Stevens and Cuthill 2007, Turner, White et al. 2009), we conclude that it is necessary for researchers to demonstrate that signalers benefit from less

detectable communication before they declare that a signal is private. In part III, we discuss how signals that are less apparent to potential eavesdroppers than intended receivers are not necessarily private, as they may contain information detectable through other sensory channels. In part IV, we describe how researchers may manipulate potential eavesdroppers to demonstrate that a signal that is less apparent to potential eavesdroppers than it is to intended receivers actually functions as protected private communication. We argue that it is important to demonstrate that a proposed protected private signal's reduced detectability is adaptive, i.e. that reduced detection by potential eavesdroppers benefits the signaler. To do this, researchers need to ask a question fundamentally different from those asked previously. Instead of only asking what a potential eavesdropper can detect, we need to ask *if a potential eavesdropper could detect the signal more clearly (up to as clearly as an intended receiver), would the signaler face fitness consequences?*

4.2 Constraint on potential eavesdroppers

4.2.1 Evolving extreme-wavelength sensitivity

Many researchers have suggested that signals in the ultraviolet (<400 nm; UV) or red (>570 nm) portions of the UV-visible spectrum are protected private signals (Denton, Gilpinbr et al. 1970, Hinton 1976, Guilford and Harvey 1998, Cummings, Rosenthal et al. 2003, Siebeck 2004, Michiels, Anthes et al. 2008, Bybee, Yuan et al. 2012). In cases where signalers and eavesdroppers have photoreceptors with peak sensitivities

to different wavelengths of light (referred to as λ_{max}), such extreme-wavelength signals can be used for protected private communication if the signal has smaller differences in luminance (achromatic contrast) or color (chromatic distance) when viewed by potential eavesdroppers than when viewed by an intended receiver (Figure 12).

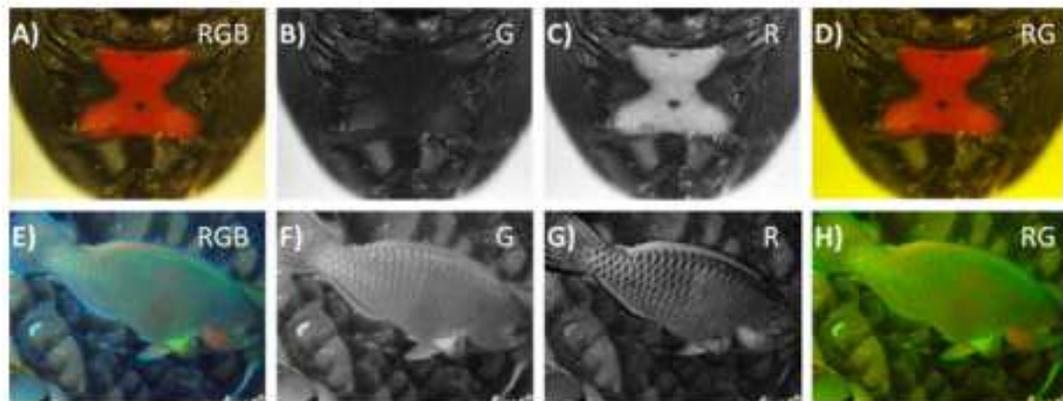


Figure 12: Examples of a possible luminance-private and color-private signal viewed by different photoreceptor classes and combinations. Letters on the top left of each image indicate photoreceptor classes present (R = red, G = green, B = blue). Top panels (a–d) A possible luminance-private channel, the hourglass of a southern black widow spider (*Latrodectus mactans*). Note that shifting the peak sensitivity of a green photoreceptor (b) to longer wavelengths (c) reveals the signal. The color information (d) largely duplicates the achromatic information. Bottom panels (e–h) A possible color-private channel demonstrated by the surf parrotfish (*Scarus rivulatus*). Note that shifting a green photoreceptor (f) to longer wavelengths (g) does not reveal all of the information found in (h) and (e). In comparison, dichromatic vision (h) makes the patterning of the fish obvious to a viewer. Adding a third photoreceptor class (e) reveals even more spatial information. a and e are unmodified images. Other images were modified in Adobe Photoshop by removing the relevant channels. Because camera sensors generally have a much narrower spectral absorbance curve than biological photoreceptors, the effects of privacy are exaggerated by the images. The intent of the figure is to show the differences in the evolutionary pressures imposed on potential eavesdroppers, and not to suggest that formal modeling suggests that either of the examples used are actually private signals.

Whether a protected private signal has reduced detectability predominantly via luminance or color determines how a potential eavesdropper's visual system would need to evolve in order to better detect the signal. Because the luminance of a signal is often related to its color, many hypothesized private signals provide luminance cues that an intended receiver can detect more clearly than a potential eavesdropper. In this case, a lineage of potential eavesdroppers only needs to evolve a single appropriately sensitive photoreceptor class to crack the private signal (Fig. 1a–d). If a private signal has a similar color to its background, such as a UV marking on a violet field or an orange spot on a yellow wing, the signal cannot be detected through luminance differences. In such cases, a potential eavesdropper needs to be able to discern the color of the signal in order to detect it. To detect a color-private signal, a potential eavesdropper would need two sets of photoreceptors that differ in peak sensitivity, yet are simultaneously sensitive to both signal and background (Fig. 1e–h). Evolving two sensitive photoreceptor classes usually involves opsin genes that duplicate and then diverge so that the photopigments they encode have different spectral sensitivities (Yokoyama 1994, Dulai, von Dornum et al. 1999, Yokoyama 2000, Spaethe and Briscoe 2004, Parry, Carleton et al. 2005). Alternatively, previously existing opsins may be co-opted for new functions (Plachetzki and Oakley 2007).

Spectral sensitivity can be shifted by relatively small evolutionary changes (Table 4). For example, single amino acid substitutions can shift the spectral sensitivity of

opsins enough to change the perception of some protected private signals in the UV or red wavelengths. In birds (415-368 nm; Yokoyama, Radlwimmer et al. 2000) and fruit flies (414-359 nm; Salcedo, Zheng et al. 2003), a single amino acid substitution can shift peak sensitivity into the UV and possibly reveal private signals based on UV wavelengths. Likewise, in both invertebrates (e.g. Briscoe 2001, Frentiu, Bernard et al. 2007) and vertebrates (e.g. Neitz, Neitz et al. 1991, Chan, Lee et al. 1992, Yokoyama and Radlwimmer 2001), three to six amino acid substitutions can shift a green ($\lambda_{\max} = \sim 530$ nm) sensitive photopigment towards wavelengths ($\lambda_{\max} = \sim 560$ nm) that will improve red discrimination. Extreme-wavelength vision can also evolve through changes to a photoreceptor's chromophore or through the addition of filters that restrict the wavelengths of light reaching certain photoreceptors. Some animals, including the winter crayfish (*Procambarus clarkii*; Girard, 1852), firefly squid (*Watasenia scintillans*; Berry, 1911), and peacock blenny (*Salaria pavo*; Risso, 1810), combine different chromophores with the same opsin protein to shift the λ_{\max} of photoreceptors by 20–35 nm (Table 4). Animals may also use filters (e.g. lens compounds, oil droplets, or overlying photoreceptors) to alter the spectral sensitivity of photoreceptors (Bowmaker and Knowles 1977, Douglas and Marshall 1999). As with changes to opsin proteins or chromophores, changes in filters may reveal private signals by shifting a photoreceptor's λ_{\max} to the red or UV portions of the visible light spectrum (Table 4).

Table 4: Changes in opsin, chromophore, or filter can result in a shift to red sensitivity, while changes to opsin or loss of a filter can result in UV vision. V = violet, G = green, R = red. λ_{\max} and λ_{\max}' represent two different sensitivities found in (1) experimental mutants, (2) different photoreceptor classes in the same species or (3) different species.

Type	λ_{\max}	λ_{\max}'	Note	species	reference
Opsin Change					
Single amino acid substitution	415	369 (1)	V-UV switch	<i>Gallus gallus</i>	(Yokoyama, Radlwimmer et al. 2000)
Single amino acid substitution	347	420 (1)	V-UV switch	<i>Drosophila melanogaster</i>	(Salcedo, Zheng et al. 2003)
Multiple amino acid substitutions	359	414 (1)	V-UV switch	<i>Mus musculus</i>	(Shi, Radlwimmer et al. 2001)
Three amino acid substitutions	530	562 (2,3)	G-R switch	<i>Homo sapiens</i>	(Neitz, Neitz et al. 1991)
Multiple amino acid substitutions	520	575 (3)	G-R switch	<i>Papilio xuthus</i>	(Briscoe 2001)
Chromophore Change					
Chromophore substitution	484	470, 500 (2)		<i>Watasenia scintillans</i>	(Seidou, Sugahara et al. 1990)
Chromophore substitution	533	567 (2)	G-R switch	<i>Procambarus clarkii</i>	(Zeiger and Goldsmith 1989)
Chromophore substitution	498	515 (2)		<i>Salaria pavo</i>	(White, Goncalves et al. 2004)
Filter					
Screening pigment	360	400 (2)	UV-V switch	<i>P. xuthus</i>	(Arikawa, Mizuno et al. 1999)
Screening pigment	500	550 (2)	G-R switch	<i>W. scintillans</i>	(Michinomae, Masuda et al. 1994)

Comparative surveys of species-rich lineages provide additional evidence that the spectral sensitivities of photoreceptors often shift in ways that can potentially reveal extreme-wavelength protected private signals. For example, four recent meta-studies (Figure 13) demonstrate that photoreceptors that are primarily sensitive to UV or red light can be found in birds (Frentiu, Bernard et al. 2007), crustaceans (Marshall, Kent et al. 1999), fish (NESCent working group on the evolution of vertebrate visual systems), and insects (Briscoe and Chittka 2001). UV vision ($\lambda_{\max} < 400$ nm) is also found in all five classes of vertebrates [amphibians (*e.g.* Deutschlander and Phillips 1995); birds (reviewed in Frentiu and Briscoe 2008); fish (reviewed in Losey, Cronin et al. 1999); (*e.g.* Jacobs, Neitz et al. 1991) and reptiles (*e.g.* Ammermuller, Itzhaki et al. 1998)]. Further, researchers have shown that UV sensitivity has been lost or gained at least eight times in birds alone (Carvalho, Cowing et al. 2007, Odeen, Hastad et al. 2011). UV vision is also common in invertebrates, as it is found in most insects (Briscoe and Chittka 2001), some arachnids (*e.g.* Devoe 1975, Walla, Barth et al. 1996), certain crustaceans (Frank and Case 1988, Marshall, Kent et al. 1999, Frank, Johnsen et al. 2012), the giant clam *Tridacna maxima* Röding, 1798 (Wilkens 1984) and the annelid worm *Torrea candida* Delle Chiaje, 1841 (Wald and Rayport 1977). Similarly, red-sensitive photoreceptors ($\lambda_{\max} > 550$), although less common, occur in all vertebrate classes, including amphibians (Perry and Mcnaughton 1991), birds (Frentiu and Briscoe 2008), fish (Loew and Lythgoe 1978), mammals (Neitz, Neitz et al. 1991), and reptiles (Loew, Fleishman et al. 2002). Red-

sensitive photoreceptors are also found in numerous invertebrates, such as insects (Briscoe and Chittka 2001), certain arachnids (Yamashita and Tateda 1976), certain crustaceans (Marshall, Kent et al. 1999), and annelids (Wald and Rayport 1977).

Evidence thus suggests that a wide range of metazoans see in the extreme-wavelengths and that potential eavesdroppers may face few lineage-specific evolutionary constraints to detecting extreme-wavelength protected private signals.

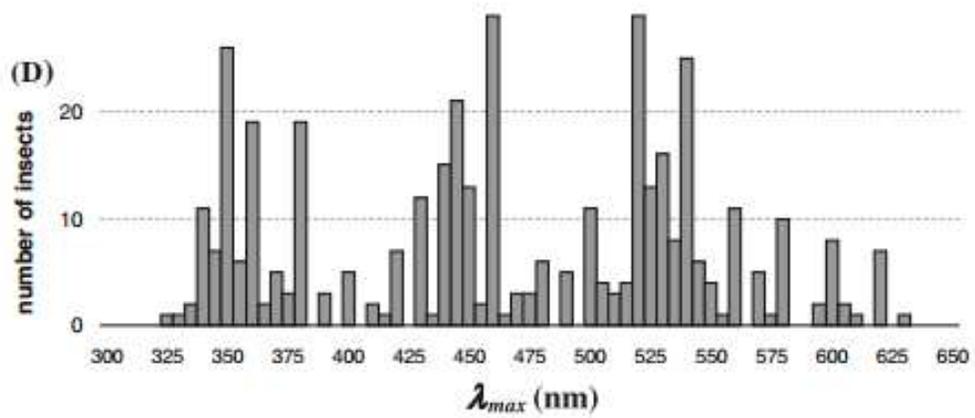
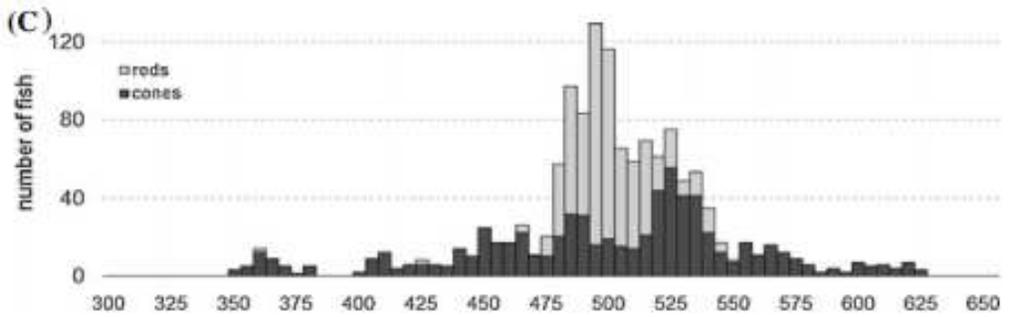
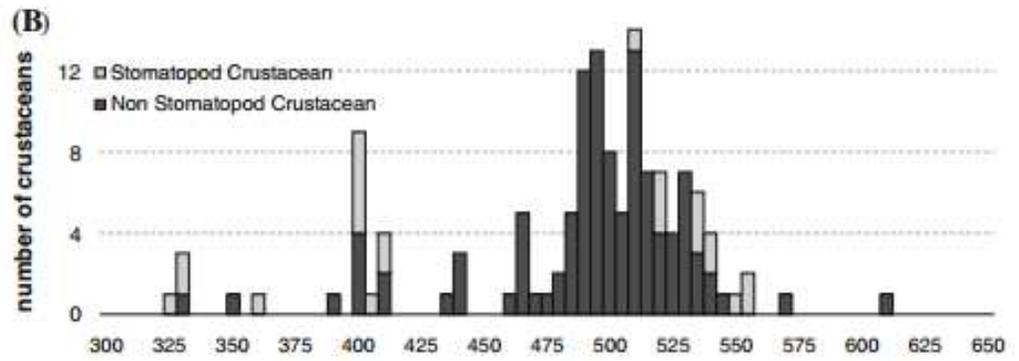
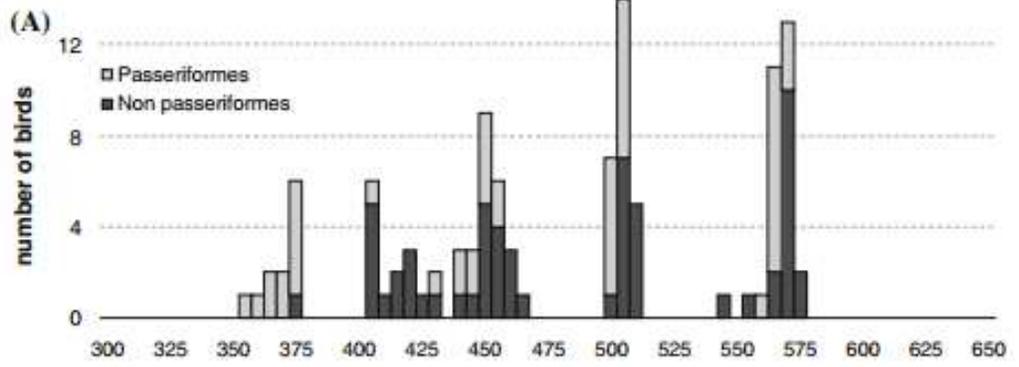


Figure 13: The known λ_{\max} values for photoreceptors from: a birds, b crustaceans, c fish and d insects. Note that within a–d many pigments are clumped around specific spectral sensitivity values; however great variety exists. Estimates of peak wavelength values are based on a variety of methods, some of which include pre-receptor filtering while others do not. In b, because of their great number of pigments, only the minimum and maximum photopigment were graphed for each species of stomatopod crustacean. Number of photoreceptor classes = (a) 107 (b) 133 (c) 1317 (d) 403.

In certain cases, researchers have shown that separate eavesdropping lineages have cracked the same protected private signal through different mechanisms. One case study involves the detection of the bioluminescence produced by three separate genera of predatory dragonfish (*Aristostomias*, *Malacosteus*, and *Pachystomias*). These species produce far red ($\lambda_{\max} \approx 700$ nm) bioluminescence that may function both as a protected private signal between conspecifics and as a private searchlight to detect prey in the deep sea where red light is uncommon (Denton, Gilpinbr et al. 1970, Levine, Lobel et al. 1980, Widder, Latz et al. 1984, Partridge and Douglas 1995). However, more than 40 years after a private function for the far red bioluminescence of dragonfish was first suggested, researchers have found evidence that at least two different fish species preyed upon by dragonfish have possibly cracked the private signal. The first species, *Myctophum nitidulum* (Garmin 1899), has redshifted a visual pigment (to a λ_{\max} of 522 nm) via a chromophore substitution coupled with a change in the opsin protein (Hasegawa, Sawada et al. 2008). A visual pigment maximally sensitive to 522 nm light is not ideal for detecting the far red bioluminescence of dragonfish, but models show that the red-sensitive photoreceptors can detect far red bioluminescence at distances up to

one meter away (Turner, White et al. 2009). The second potential eavesdropper species, *Bolinichthys longipes* (Brauer 1906), detects far-red bioluminescence using the same mechanism employed by the dragonfish themselves—a chlorophyll-derived sensitizing pigment with a λ_{\max} of ~670 nm (Turner, White et al. 2009).

Over evolutionary time, the spectral sensitivities of photoreceptors appear to be relatively unconstrained within the UV-visual spectrum. However, the long-wavelength end of this spectrum deserves special attention: no known photoreceptors have a λ_{\max} greater than ~630 nm, even though long-wavelength light is abundant in many habitats (Johnsen 2012). This upper limit on spectral sensitivity may be due to thermal noise—the activation of photoreceptors by heat and not light (De Vries 1943, Barlow 1956, Ala-Laurila, Donner et al. 2004). In general, there is an inverse relationship between the λ_{\max} of a photopigment and its activation energy: photopigments sensitive to long-wavelength light have a relatively low activation energy and are thus more susceptible to thermal noise (Ala-Laurila, Donner et al. 2004, Ala-Laurila, Pahlberg et al. 2004, Pahlberg, Lindstrom et al. 2005, Luo, Yue et al. 2011). Because animals cannot discriminate between photoreceptors triggered by light and photoreceptors triggered by heat, thermal noise may make photoreceptors sensitive to long-wavelengths too unreliable for vision.

Concerning protected private communication, we hypothesize that animals differ in their ability to limit thermal noise (Ala-Laurila, Pahlberg et al. 2004) in ways

that influence whether or not they can gather information about long wavelength light. If we are correct, signalers may be able to exploit differences between themselves and potential eavesdroppers in ways that make protected private communication stable over evolutionary time. We hypothesize that one species could detect long-wavelength light that another species could not due to differences in any of the following: (1) opsin protein structure (Ala-Laurila, Donner et al. 2004, Ala-Laurila, Pahlberg et al. 2004, Luo, Yue et al. 2011), (2) body temperature [hotter eyes generally have more thermal noise (Ashmore and Falk 1980, Aho, Donner et al. 1988, Aho, Donner et al. 1993, Reilly and Thompson 2007)], or (3) eye size [smaller eyes are generally more susceptible to thermal noise because they have worse signal to noise ratios (Warrant and Locket 2004)]. We predict that small eyes operating at high temperatures will have the most trouble with thermal noise and will thus be the least likely to contain photoreceptors sensitive to long-wavelength light. However, more work is needed to test this hypothesis and to examine if temperature-related constraints on spectral sensitivity may be exploited for protected private signals involving far-red light.

4.2.2 Evolving polarization sensitivity

Signals involving polarized light are potentially effective for protected private communication because photoreceptor morphology differs between invertebrates and vertebrates in ways that give only invertebrates an innate potential for polarization sensitivity (Figure 19 in Appendix D; Moody and Parriss 1961, Land and Nilsson 2002,

Horváth and Varjú 2004) Based on typical photoreceptor geometry, researchers have hypothesized that organisms may be able to signal privately to polarization-sensitive invertebrate receivers without polarization-insensitive vertebrates being able to evolve the ability to detect the signal (Shashar, Rutledge et al. 1996, Mathger and Hanlon 2006, Douglas, Cronin et al. 2007, Mathger, Shashar et al. 2009, Brady and Cummings 2010).

Polarization sensitivity appears to be far more common in invertebrates than vertebrates, but it has been reported that certain vertebrates [e.g. amphibians (Adler and Taylor 1973, Auburn and Taylor 1979), birds (Kreithen and Keeton 1974, Muheim 2011), and fish (Waterman and Forward 1970, Kamermans and Hawryshyn 2011)] may detect the polarization of light through a variety of mechanisms (Table 5). Despite these reports, researchers have yet to reach a consensus on how many vertebrates are polarization sensitive because behavioral investigations of polarization sensitivity are prone to intensity artifacts that may cause false positive reports (Horváth and Varjú 2004), and research on polarization signals has typically been biased towards invertebrates. Polarization sensitivity has, however, been demonstrated convincingly in at least one vertebrate family (*Engraulidae*; Fineran and Nicol 1976, Flamarique, Hawryshyn et al. 1998, Flamarique and Harosi 2002). Because some of the mechanisms through which vertebrates may obtain polarization sensitivity require relatively complicated optics, we predict that vertebrates may pay higher functional costs for polarization vision than invertebrates. In this sense, vertebrates may not be constrained

Table 5: Although invertebrates often possess an innate polarization sensitivity that vertebrates lack, vertebrates have been reported to detect polarization by a variety of different mechanisms. In comparison to the most common mechanism used by invertebrates to detect polarized light (I), many of the potential vertebrate mechanisms may impose higher functional costs

Proposed mechanism	Proposed in	Possible additional functional costs relative to mechanism I	Reference
I) Alignment of opsins (and thus the associated chromophores) within fingerlike projections of cell membrane	invertebrates		(Moody and Parriss 1961)
II) Polarizing (<i>i.e.</i> dichroic) filters between incident light and photoreceptors	universal	negligible	(Johnsen 1994)
III) Tilted photoreceptors that mimic the mechanism found in invertebrates	anchovies (<i>Anchoa</i>)	requires more retinal space	(Fineran and Nicol 1976)
IV) Polarization-dependent reflections within double cones	amphibians, birds, fish, reptiles	requires more retinal space, heightens metabolic demands	(Cameron and Pugh 1991)
V) Polarization-dependent reflections within double cones that direct light toward a third photoreceptor	rainbow trout (<i>Oncorhynchus mykiss</i>)	requires more retinal space, heightens metabolic demands, reduces photon-catch	(Flamarique, Hawryshyn et al. 1998)
VI) Alignment of opsins in photoreceptors, presumably mediated by cytoskeletal components	universal	negligible	(Roberts, Porter et al. 2011)

from evolving polarization sensitivity, but constraints on photoreceptor morphology may lead to greater costs of polarization sensitivity in vertebrates than in invertebrates (see 4.3.1; Table 5).

4.2.3 Conclusion

In this section, we suggested that published studies find few taxon-based constraints that would keep potential eavesdroppers from evolving the ability to detect common visual protected private signals. Many lineages of animals have separately evolved both red and UV vision, and polarization sensitivity has been documented in a wide range of invertebrates and satisfactorily demonstrated in at least one vertebrate family. Thus, we suggest that although potential eavesdroppers could be constrained from detecting a visual private signal in certain cases, many potential eavesdroppers may be able to evolve the ability to detect certain proposed private communication channels.

4.3 Functional costs of detecting protected private signals

Regardless of evolutionary constraints, a signal may remain private over evolutionary time if the ability to detect it is associated with net functional costs to potential eavesdroppers. In general, new visual abilities come with functional costs: improvements to one aspect of vision generally cause detriments to others. For example, the evolution of color vision can lower sensitivity (Clarke 1936, Munz 1958), coarsen spatial resolution (Lind and Kelber 2011), and require compensation for longitudinal

chromatic aberration (Blest, Hardie et al. 1981, Kroger, Campbell et al. 1999, Kroger and Gislén 2004). Some of these costs are reduced in larger eyes. With a larger eye, certain visual abilities can be added or improved without detriment to others (Land and Nilsson 2002). However, larger eyes may require (among other demands) larger optical lobes for information processing, a larger head, and increased amounts of ATP (Laughlin, van Steveninck et al. 1998, Laughlin 2001, Niven, Anderson et al. 2007). Thus, potential eavesdroppers likely face functional costs if they evolve the ability to detect a protected private signal. If these costs outweigh the benefits a potential eavesdropper would gain from detecting a signal, the signal may remain private over evolutionary time.

Three particular functional costs merit further discussion because they may tend to pose a greater evolutionary hurdle for potential eavesdroppers than for intended receivers, and thus may be exploited for protected private communication. These costs are associated with: (1) vertebrates detecting polarized light; (2) UV vision; and (3) animals detecting signals that rarely occur in nature, such as circularly polarized light.

4.3.1 Costs of polarization vision may vary by evolutionary lineage

Vertebrates may pay a higher cost to detect private polarization signals because polarization sensitivity may rely on more complicated optics in vertebrates than in invertebrates (see 4.2.2.) These heightened costs may include increased retinal area, lower photon-catch, and higher metabolic demand (Table 5). However, if vertebrates can

achieve polarization sensitivity by aligning photopigments along a common e-vector in their photoreceptors (Roberts, Porter et al. 2011) or by placing polarization filters in front of certain photoreceptors (Johnsen 1994), the costs of polarization sensitivity may be similar for both vertebrates and invertebrates. No vertebrate has yet been shown to achieve polarization sensitivity by these means, making the lineage specific costs associated with evolving polarization sensitivity an intriguing issue for future research.

4.3.2 UV-specific costs

UV-specific costs have led some researchers to suggest that UV light may be used as a protected private signal more often than red light (Cummings, Rosenthal et al. 2003, Siebeck 2004). Evolving UV vision not only imposes the general costs associated with adding a new color channel, but may incur additional costs associated with radiation damage and UV-scattering (Leech and Johnsen 2009). For example, costs associated with color vision, such as longitudinal chromatic aberration, may be heightened for detecting UV light when compared to detecting light with longer wavelengths because UV light tends to be farther away from the peak sensitivity of most animal's photoreceptors than long wavelength light. These costs are best illustrated by animals such as primates (Wood and Truscott 1993) and some fish (Siebeck and Marshall 2001) that have UV-absorbing filters that prevent light with wavelengths shorter than ~400 nm from entering their eyes. These filters are even found in cases where animals possess photoreceptors

with some sensitivity to UV radiation (such as the blue-sensitive cones in the human eye).

UV radiation can damage eyes by inhibiting protein synthesis and causing cataract formation (Zigman and Bagley 1971, Ham, Mueller et al. 1976, Collier, Waldron et al. 1989, De Mora, Demers et al. 2000). However, such damage requires substantial amounts of UV radiation, so UV costs are highest in terrestrial or shallow aquatic habitats where UV radiation is abundant. Because UV damage is cumulative, animals with longer lifespans likely suffer more from UV radiation than short-lived animals. This might allow an individual to use a UV signal to communicate privately with short-lived receivers without longer-lived potential eavesdroppers evolving the ability to detect it. In cases where top predators live longer than other species (Shuter, Lester et al. 2005), this may allow species lower on the food chain to use a stable protected private signal against top predators.

In addition to radiation damage, the physical properties of UV light may reduce eavesdropping in two distinct ways. First, in certain environments, UV light is attenuated more quickly than visible light. In these environments, UV signals may degrade quickly with distance; thus, individuals could communicate with organisms nearby without fear of those farther away detecting the signal (Siebeck 2004). Second, if UV light enters the eye, the visual contrast of all signals may decrease if the photoreceptors of the eye also receive spacelight—i.e. photons originally emitted from

the signal's background that were then scattered into the path of the signal (e.g. Fig. 5 in Johnsen 2007). If an eye does not restrict UV light from reaching the retina, all photoreceptors would respond to UV spacelight because all photoreceptors have a secondary sensitivity peak in the UV in addition to their primary sensitivity peak (Govardovskii, Fyhrquist et al. 2000).

In certain habitats, however, the UV-specific contrast attenuation described above may not be significant over distances at which potential eavesdroppers can detect signalers. For example, if an animal in dry air views a target 100 meters away, similar amounts of UV (97.5 %; 400 nm) and long-wavelength photons (98.8 %; 700 nm) reach its eyes (Bass and Optical Society of America. 1995). In this case, it is likely that the slightly increased attenuation of UV light is irrelevant. In some aquatic environments, the wavelength-dependent bias in attenuation may be greater, both because attenuation at all wavelengths is greater and because light absorption is often far higher at UV wavelengths than at other wavelengths. Because natural waters vary in how much light they scatter and absorb, it is necessary to examine each environment separately before assuming that UV signals will not propagate as far as longer-wavelength signals. In some cases, underwater contrast may actually be higher at UV wavelengths due to the spectrum of the illumination, the spectral reflectance and size of the signal, and—for viewing in directions other than horizontal—the wavelength dependence of the

difference between the diffuse and beam attenuation coefficients (Lythgoe 1968, Lythgoe and Partridge 1991, Marshall 2000, Johnsen 2002).

4.3.3 Net costs vary by the rarity of signal properties

Evolving the ability to detect a particular protected private signal may provide additional benefits for an eavesdropper. For example, if a predatory bird evolves UV vision in order to better detect the UV plumage of a prey bird, this new ability may also benefit the predatory bird by making the urine of rodents (Viitala, Korpimaki et al. 1995) and the plumage of other birds (Hastad, Victorsson et al. 2005) more obvious.

Conversely, a protected private signal that uses a form of information found rarely in nature may minimize the net benefit a potential eavesdropper may gain from evolving the ability to detect it. Thus, we hypothesize that the ability to detect a novel sensory signal might be relatively costly to an eavesdropper when it cannot be used for other tasks.

Circularly polarized light is one example of a signal that is rarely encountered in nature. Circularly polarized light has only been identified in light reflected from the exoskeletons of certain arthropods, such as stomatopod crustaceans and scarab beetles (Neville and Caveney 1969, Chiou, Kleinlogel et al. 2008), the bioluminescence of some *Photuris* fireflies (Wynberg, Meijer et al. 1980), and in minor amounts at water–air interfaces (Horváth and Varjú 2004). Potential eavesdroppers might be less likely to evolve sensitivity to circularly polarized light because this ability lacks value for other

visual tasks. To date, researchers have only demonstrated sensitivity to circularly polarized light in two groups of animals—stomatopod crustaceans (Chiou, Kleinlogel et al. 2008) and scarab beetles (Brady and Cummings 2010)—and have suggested a protected private communication function for circularly polarized light in both.

4.3.4 Conclusion

If a lineage of potential eavesdroppers improves their detection of a private signal, they likely will increase their functional costs relative to prior to being able to detect the private signal. While evolving the ability to detect any private signal may increase functional costs, the costs associated with polarized signals, UV signals, and signals found rarely in nature (such as circularly polarized light) may provide protection against some potential eavesdroppers evolving the ability to detect a protected private signal. However, increased costs in these three cases may not always apply, and researchers should examine their specific system with care.

4.4 Multimodality of protected private signals

Regardless of the constraints and functional costs associated with evolving the ability to detect a protected private signal, certain hypothesized private signals may not be private because they provide eavesdroppers with detectable information via other sensory modalities. When producing an acoustic signal, for example, a signaler may also move or vibrate (Parri, Alatalo et al. 1997, Taylor, Buchanan et al. 2007). Even if an eavesdropper cannot detect the acoustic signal, the byproducts of signaling may give an

eavesdropper all the information that it needs to detect the signaler. This scenario is especially likely if the eavesdropper only needs to locate a signaler and does not need to interpret other pieces of information carried by the signal. While in some cases the signal may be much more obvious than its byproducts, in other cases researchers need to treat a signal as multimodal and examine all channels through which potential eavesdroppers may detect both the signal and the act of signaling.

Extreme-wavelength signals are one example of a multimodal signal. Like all color signals, extreme-wavelength signals may provide information about both luminance and color. Their privacy will depend on both parameters: an extreme-wavelength signal may be private when either its luminance contrast or color contrast is lower to a potential eavesdropper than an intended receiver (Figure 12). In songbirds, for example, Stevens and Cuthill (2007) noted that although the UV plumage regions modeled by Hastad et al. (2005) provide color differences that may be more apparent to conspecifics than potential predatory eavesdroppers, the UV plumage also provides luminance differences that are easily detectable by non-UV sensitive predators. Because different colors may provide different luminance levels, we need to simultaneously consider the color and luminance of hypothesized protected private signals; just because a potential eavesdropper cannot discriminate a certain color does not mean they cannot detect a signal via luminance differences.

Like extreme-wavelength signals, polarized signals may be detected through multiple channels: the structures that produce polarized signals (Land 1972, Seago, Brady et al. 2009) often simultaneously produce saturated colors that may provide potential eavesdroppers with detectable color and luminance cues (e.g. Figure 14; Sweeney, Jiggins et al. 2003, Douglas, Cronin et al. 2007). If polarized signals are used by signalers to reduce the likelihood of detection by potential eavesdroppers, these polarized patterns must not co-occur with detectable color or luminance patterns. At the very least, the color or luminance patterns should be less obvious to potential eavesdroppers than the polarization patterns.

The neotropical butterfly *Heliconius cydno* (Doubleday, 1847) is one example of an animal with a polarized signal that potentially provides information in multiple channels. *H. cydno* likely uses both its color (Mallet 1993) and polarization (Sweeney, Jiggins et al. 2003) patterns as a signal to mates (Figure 14 B); however, researchers have suggested that the polarization component may function as a private signal that predatory birds cannot detect (Douglas, Cronin et al. 2007). To test a single case of the chromatic components of polarized signals, we modeled the polarization distance, color distance, and luminance contrast between two regions of the wings of the neotropical butterfly *H. cydno* (For full methods and definitions see Appendix E). If the polarized

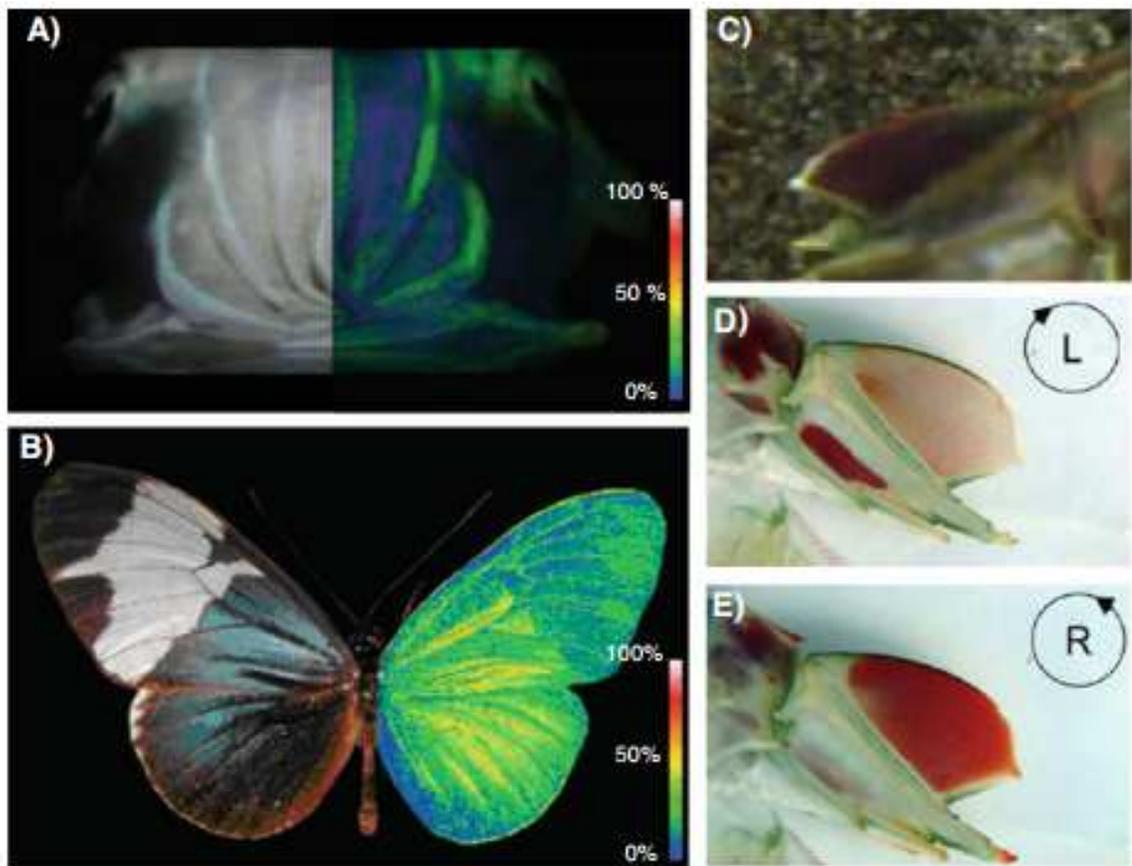


Figure 14: The color components of polarized signals. Images of a the cephalopod *Sepia officinalis* and b the butterfly *H. cydno*. The left hand side of each image shows a color photograph of the animal, while the right is a false-colored polarization image that shows degree of polarization. c–e The circularly polarized keel of a stomatopod crustacean, viewed under normal conditions and left- and right-handed polarized light. Note that in all three animals strong chromatic or achromatic components accompany polarized reflectance. Images from a (Chiou, Mäthger et al. 2007), b (Douglas, Cronin et al. 2007), c–e (courtesy of Christine Huffard)

signal is private and reduces detection by potential eavesdroppers, we would expect the polarization pattern to be more distinct than both the color and luminance patterns.

However, when viewing isolated components of *H. cydno's* appearance (Figure 15), we

find that the wing's polarization pattern (Figure 15b; polarization distance = .276) is accompanied by color patterns (Figure 15c; color distance = .176) and luminance differences (Figure 15d; ~2.5 times brighter). These color and luminance differences make it unlikely that *H. cydno* utilizes polarized reflectance as a protected private signal.

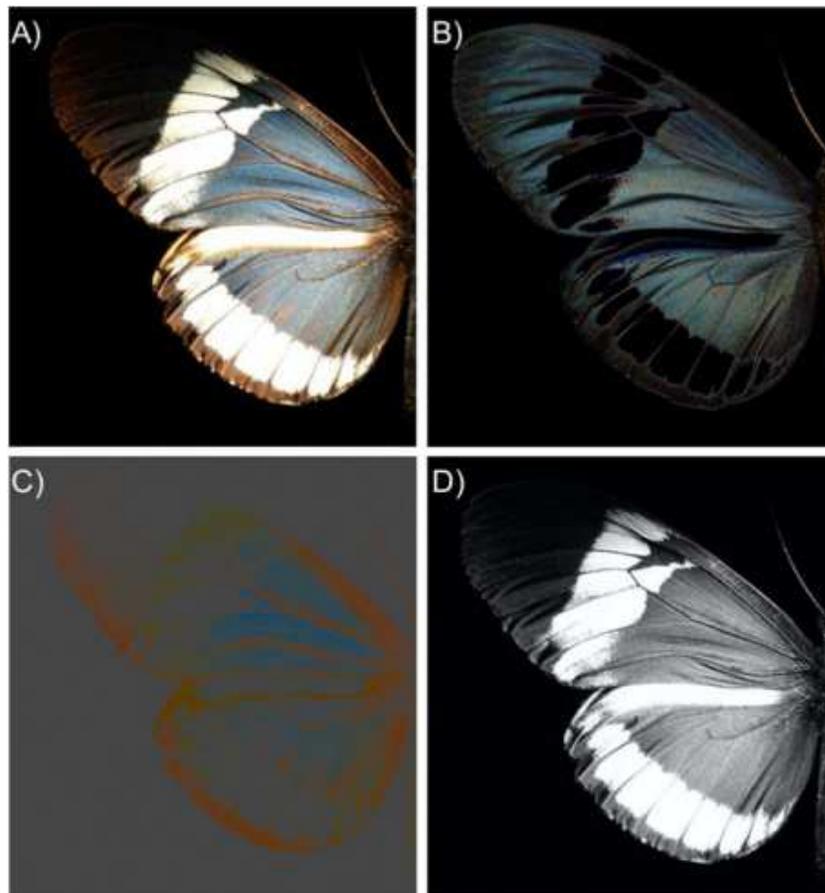


Figure 15: Multiple channels of reflectance information in *H. cydno*. a An unmodified wing of *H. cydno*. b Polarization image of *H. cydno* multiplied by the normal image to show degree of polarization as a function of color. Full polarization views as white, 0 % polarization is black. c Color-only image where achromatic information has been removed. d Achromatic-only image (black–white). Note that the wing's polarization differences (b) strongly correlate with achromatic differences (d) that would be detectable by a polarization-blind eavesdropper. Original photo adapted from (Sweeney, Jiggins et al. 2003)

The example of *H. cydno* suggests that some polarization-blind eavesdroppers may be able to detect certain polarized signals. In order to signal privately via polarized signals, an animal needs to match its polarized signal's chromatic and achromatic reflectance to the background. For example, the scarab beetle *Chrysina gloriosa* (LeConte 1854), has a green exoskeleton that produces polarized reflections that may spectrally match the juniper foliage on which this animal lives (Brady and Cummings 2010). While the privacy of some polarized signals may be limited to specific backgrounds, certain cephalopod mollusks (e.g. squid, octopus, and cuttlefish) possess anatomical features that could produce private polarized signals against a variety of backgrounds. Many cephalopods have two different layers of cells that control their dermal coloration. The inner layer, packed with iridophores, reflects polarized light at short wavelengths (Mathger, Shashar et al. 2009). These iridophores are covered by an outer cell layer that contains chromatophores—pigment containing sacs that expand and contract via neural control (Florey 1969). Notably for protected private communication, light passing through the chromatophores changes in color, but not polarization (Mathger and Hanlon 2006). Theoretically, a cephalopod's iridophores could produce polarized signals whose color could be altered by the chromatophores to match different substrates (Mathger, Shashar et al. 2009). While it is an intriguing hypothesis, researchers currently lack behavioral data demonstrating (1) that cephalopods actually mask the chromatic

components of polarized signals and (2) that the ability to mask polarized signals in this manner is adaptive.

4.5 Demonstrating an adaptive value for reduced detection of a signal

In order to show that a signal is private, we need to demonstrate that its secrecy is adaptive. Here we discuss four ways to test whether the reduced detection of a proposed private signal benefits a signaler. These methods include: (1) manipulating the signal; (2) manipulating a potential eavesdropper's perception of the signal; (3) removing potential eavesdroppers from a population of signalers and measuring whether selective pressures on the signaler decrease; or (4) manipulating the numbers of potential eavesdropper in different populations of signalers and comparing how the signal changes over time between populations (Table 6). Note that for many of these methods, researchers need to follow signalers or potential eavesdroppers over long periods of time. The exact lengths of time required will depend on whether the expected changes occur via phenotypic plasticity or are evolved responses.

Table 6: Researchers can use a variety of methods to demonstrate that the reduced detection by eavesdroppers of a hypothesized protected private signal is adaptive.

Method	Expectation if signal is private	Pros	Cons
1) Manipulate the signal	More conspicuous signals negatively impact factors associated with a signaler's fitness.	Signals can be switched from one modality to another. Can fully demonstrate that the privacy of a signal is adaptive.	May give false negative results if potential eavesdroppers do not respond to an unfamiliar modified signal, or do not have enough time to evolve the ability to detect it.
2) Manipulate the potential eavesdropper's perception	If a potential eavesdropper and intended receiver have the same sensory abilities, the signaler's fitness will decrease.	Can demonstrate an adaptive function for a private signal.	Requires knowledge of the underlying sensory genetics of the potential eavesdropper.
3) Remove the potential eavesdropper	A signal will become more genetically and/or phenotypically diverse when potential eavesdroppers are absent.	May be less intensive than methods 1 and 2.	May give false negative results if other forces are maintaining the signal's form. Dependent on generation time of signaler. Cannot fully rule out other factors responsible for a signal's form. Pleiotropy may lead to false results.
4) Compare and manipulate different populations containing signalers	Signals will be more obvious to potential eavesdroppers in populations where potential eavesdroppers are absent.	May be less intensive than methods 1 and 2.	Cannot fully rule out other factors responsible for a signal's form. Pleiotropy may lead to false results.

Via signal manipulation experiments, we can alter a proposed private signal to make it more obvious to a potential eavesdropper. Instead of only asking what a potential eavesdropper can detect, manipulating the signal asks: if a potential eavesdropper could detect a signal more clearly (up to as clearly as an intended receiver), would the signaler face fitness consequences? Depending on the modality of the signal and the sensory processing of the potential eavesdropper, the exact methods will vary. If a signaler suffers from increased eavesdropping after its signal has been manipulated, we would conclude that the signal has an adaptive private value. However, the above methods may give us false negative results: potential eavesdroppers may need time to either learn, or evolve, the behavioral capacity (independent of physiology) to use the information present.

If signals in differing modalities are processed in similar ways, we could convert a signal from one modality to another. For example, we could utilize the similarities between polarization and color processing to convert a polarized signal into a color signal. This is feasible because it appears that color and polarization processing are similar in that both can be represented as the responses of two or more photoreceptor classes to a stimulus (Bernard and Wehner 1977, Glantz 2001, Kleinlogel, Marshall et al. 2003). However, differences between color and polarization processing could alter the results. Additionally, no signal is completely isolated from its environment. A polarization signal may contrast highly with its background relative to other

polarization signals in the scene. If this polarization signal is then converted into a chromatic one, however, the new signal may have a low contrast relative to the contrasts occurring in the environmental background.

Instead of manipulating signals, researchers may soon be able to modify a potential eavesdropper's ability to detect a particular signal. For example, molecular biologists can already manipulate the spectral sensitivities of photopigments through targeted alterations to the amino acid sequences of opsin proteins (*e.g.* Yokoyama and Radlwimmer 2001). Given decreased costs associated with gene discovery and improved methods for altering gene expression *in vivo*, it soon may be possible to create potential eavesdroppers with sensory abilities that mimic those of intended receivers. Doing so would require: (1) identifying the genes in a potential eavesdropper responsible for detecting a particular signal; (2) modifying these genes so that the resulting proteins have the desired functional characteristics; and (3) expressing the new genes in place of the old ones in living individuals. These modified eavesdroppers could then be used in behavioral experiments to determine if signalers suffer fitness consequences when their signals are viewed by potential eavesdroppers with sensory capabilities similar to intended receivers.

In a third method for testing whether the reduced detection of a particular signal by eavesdroppers is adaptive, we propose that researchers track how signals change over time when potential eavesdroppers are removed from an environment. If a signal is

private and relevant eavesdroppers are absent, we have removed the selective pressure on the signal to remain less detectable. Without this pressure, genetic drift (or natural selection if the private qualities of a signal are associated with fitness costs) and differences in expression may cause signals to become more detectable than they were when potential eavesdroppers were still present. Within a population, we would also expect that microhabitats without potential eavesdroppers contain a greater variation in signals than environments where potential eavesdroppers are still present (McShea and Brandon 2010). However, this method may fail to support an existing private function for a signal if other selective pressures (such as sexual selection) maintain the signal's form. Additionally, for many animals with long generation times, researchers may need to observe populations (and constantly ensure potential eavesdroppers are still not present) for longer than is experimentally feasible in order to detect a change in a potentially private signal.

Finally, researchers can combine a comparative approach with potential eavesdropper manipulation to provide evidence that a particular signal is private (*e.g.* Endler 1980). This fourth experimental method requires both (1) comparing natural populations to establish the private function of a signal in nature, and (2) experimental manipulations that examine whether potential eavesdropper occurrence, and not another factor, is the mechanism responsible for the reduced detectability of a signal. For example, if the occurrence of an eavesdropping predator varies between isolated

populations of signalers, we would first compare signals between isolated populations. On average, we would expect signals to be less detectable to potential eavesdroppers in populations where potential eavesdroppers are present than where they are absent. However, this method does not rule out other factors (*e.g.* temperature, other eavesdroppers, and habitat) that may differ by population and could be correlated with both the presence of potential eavesdroppers and the detectability of the signal. To partially rule out these factors, we could introduce potential eavesdroppers to some isolated prey populations. In populations where potential eavesdroppers were introduced, we would expect selection to favor signals that have reduced conspicuousness in characteristics that potential eavesdroppers can detect. This approach does not necessarily prove that a signal is private. Potential eavesdroppers may alter their environment in other ways besides eavesdropping, such as changing the total species composition (Paine 1966). Therefore, differences observed between treatments may be a result of these factors and not to reduced rates of eavesdropping.

In short, by manipulating potential eavesdroppers and/or signalers, researchers can test whether a proposed protected private signal benefits the signaler because of a reduction in detection by potential eavesdroppers (Table 6). Unfortunately, each possible method has its own drawbacks, and all of them are daunting tasks. However, we feel that the benefits of demonstrating an adaptive value for a proposed private signal outweighs these difficulties; without demonstrating that a proposed protected

private signal is actually private, we may confuse actual private signals with signals that are undetectable for non-adaptive reasons.

4.6 Conclusion

Protected private communication relies on physiological and evolutionary asymmetries between potential eavesdroppers and intended receivers. Protected private signals can remain private over evolutionary time through four different mechanisms: (1) evolutionary constraints, (2) functional costs to detection, (3) relationships between signalers and potential eavesdroppers that change faster than selection can act, or (4) a co-evolutionary arms race. If no constraints apply, a potential eavesdropper's lineage may be able to evolve the ability to detect a protected private signal. In a variety of animal taxa, studies suggest that spectral sensitivity has often changed in ways that would reveal protected private signals using UV and red wavelengths (Table 4). Similarly, studies suggest that vertebrates may be able to detect the polarization of light by a variety of mechanisms, but that some of these mechanisms may be associated with greater functional costs than those generally employed by invertebrates (Table 5).

If a potential eavesdropper evolves the ability to detect a formerly private channel, they may suffer functional costs because optical principles imply tradeoffs between color vision, polarization vision, sensitivity, and acuity. No eye can improve all visual parameters at once without increasing in size and metabolic demand. However, costs to sensory abilities are only one part of a potential eavesdropper's net fitness, and

it is the net fitness of a potential eavesdropper (along with the fitness of signalers and intended receivers) that will determine whether a protected private signal remains private over evolutionary time. Many other variables, such as the rate of occurrence of the signal and the benefit of its detection, will also determine the prevalence of protected private communication. Often, net fitness may not be experimentally measurable or may involve too many variables to be modeled with reasonable accuracy. For instance, if a falcon could detect UV light, would the benefit of detecting a song bird's UV plumage outweigh the loss of acuity from scattered light, increased UV damage to the eye, and the need to correct for additional chromatic aberration? To predict this we would need to know: (1) how often the passerine and the hawk interact, (2) the per occurrence increase in successful predation resulting from the hawk seeing the private signal, (3) the occurrence of other prey, (4) the per occurrence decrease in successful predation of other prey because of the loss of acuity from light scattering, (5) the fitness costs from UV radiation damage to the eye, and 6) the costs of shifting a violet pigment into the UV on optical sensitivity and the visual contrast of other relevant objects. Needless to say, these are daunting tasks. An alternative, possibly simpler, method is to demonstrate that an undetectable signal benefits the signaler because of its secrecy (Table 6).

It is important to note that differences in behavior, rather than physiology, can also lead to communication free from eavesdroppers. In some cases, these behaviors may be less evolutionarily labile in potential eavesdroppers than certain aspects of their

sensory physiology. For example, the fiddler crab *Uca tangeri* (Eyedoux, 1835) may use differences in microhabitat to signal to potential mates with parts of its body (*e.g.* its mouth parts or the bottoms of its claws) that are camouflaged from predators approaching from above, such as birds (Cummings, Jordao et al. 2008). It is unlikely, although not impossible, that a bird would benefit if it foraged with its head held against the ground to detect the crabs at their most conspicuous viewing angle. Similarly, some coral reef fish may use contrasting stripes that blur together to match the background when viewed by a predator at a distance (Marshall 2000). A predator could increase the distance at which it could detect such fish by evolving better spatial resolution (at the cost of either lower sensitivity or a larger eye). However, if these coral reef fish are often near intended receivers, but far from predators, the eye size required by the predator to detect its prey may incur maintenance costs that outweigh its benefits. Signalers can also switch from signaling in one modality to another as the cost-benefit ratio of these signals change with environmental conditions. For example, when bat predators pose a higher risk during the full moon, certain neotropical katydids (Family Pseudophyllinae) switch their signaling from acoustic songs to substrate vibrations (Romer, Lang et al. 2010). In order to detect the vibrations, predatory bats would have to forage by landing on each katydid's individual plant.

To date, no hypothesized protected private signal has been shown to benefit the signaler because of the signal's reduced detection by potential eavesdroppers. We do not

consider it a coincidence that two of the oldest proposed protected private communication channels, the far-red bioluminescence of dragonfish and the UV plumage of passerines, have been cracked by certain likely eavesdroppers (Stevens and Cuthill 2007, Turner, White et al. 2009). Future studies on private signaling need to demonstrate that an undetectable signal is adaptive, and not undetectable for non-adaptive reasons.

5. Conclusions and future directions

5.1 Conclusions

5.1.1 Selection and the form of an aposematic signal

My results suggest that particular aspects of black widow coloration have been under stronger selection than others. Specifically, I found that both the black widow's red-and-black color combination and macro patterning (presence of red dorsal coloration) are consistent with being under selection to be conspicuous to potential predators and inconspicuous to potential prey (Chapter 2). However the micro-patterning of the black widow's hourglass morphology showed levels of variance that suggest relaxed selection on hourglass shape (Chapter 3), including showing no evidence of regional mimicry. I hypothesize that this may be because potential predators view hourglass shape categorically rather than continuously, and do not alter their behavior towards different hourglass shapes (Chapter 3). If this is the case, then why have prey not selected hourglass shape to be minimal as it appears happened to red dorsal coloration in the North American black widows? Similarly to potential predators, differences in hourglass shape may be too small for prey to resolve (and distinguish) at behaviorally relevant distances (Land 1997). Thus while predators and prey can select for black widow hue and macro-patterning, micro-patterning is more likely to evolve under selectively neutral circumstances.

5.1.2 Black widows and private signaling

In chapter 2, I suggest that the red-and-black color combination of black widows may function as a private communication channel to avian predators while being inconspicuous to insect prey. Like other studies on private communication (*e.g.* Cummings, Rosenthal et al. 2003, Siebeck 2004, Brady and Cummings 2010), I show that the black widow coloration is more conspicuous to birds than insects. However, I do not show a selective advantage of this coloration compared to other possible color combinations (see future directions 5.2.2), and I do not examine the evolutionary possibility of insects being able to crack the private communication channel (Chapter 4). Two factors favor the ability for black widows to maintain a protected private communication channel over evolutionary times. First, as generalist predators, black widows may exert only minimal pressure on insect vision (see 4.3). Second, the prey of black widows have eyes that are smaller than bird eyes, and therefore will generally be noisier. Inherently noisier eyes may have problems evolving improved red vision because of thermal noise (see 4.2.1), which would make red vision less advantageous in black widow's insect prey. These factors combined may make the private communication channel of black widows stable over evolutionary time.

5.2 Future directions

As the visual ecology of black widows has largely been understudied prior to this thesis, there are many possible avenues of future research. Here I suggest four of particular interest.

5.2.1 Quantifying black widow vision

Because of their small eye size and reliance on other senses, this thesis has assumed that black widow vision has not influenced the evolution of black widow coloration. ERG or MSP could be used to examine the spectral sensitivity of black widows and determine their ability to distinguish their own red markings. In addition, black widow spatial resolution could be examined to determine how they are able to view each other over a variety of distances. Together these findings could confirm the possibility of black widow vision shaping black widow coloration.

5.2.2 An adaptive benefit for the black widow's private communication channel

While I suggest a private communication channel for red-and-black coloration, I did not demonstrate or quantify any adaptive advantage for the red-and-black color combination over other possible color combinations. In the field, black widow coloration could be modified to become more visible to insects such as using a white-and-black or yellow-and-black combination in addition to a sham-control red-and-black combo. Black widow webs could then be checked daily for prey carapaces to see if different color combinations would influence black widow foraging ability (see 4.5 for caveats). In

addition, black widows could be examined for any signs of changes in predation, such as web damage, missing limbs, or absent widows.

5.2.3 Hourglass shape and predator responses

My results from Chapter 3 suggest that predators may not behaviorally discriminate between different shapes of hourglasses. Using the same field method as in Chapter 2, I could present wild birds with model black widows that varied in hourglass shape. Notably, using the variation in hourglass shape already observed in Chapter 3, I could determine if any differences in hourglass shape have functional consequences by presenting model black widows with hourglasses representing the 10th, 25th, 50th, 75th and 90th percentile of the various hourglass principal components. Note that because I do not expect any differences in bird attack rate between the hourglass shapes, these experiments would require a large sample size to get reasonable statistical power.

5.2.4 Black widow coloration, relatedness, and condition

My results from Chapter 3 suggest a role for both condition and relatedness (through species type) in shaping hourglass shape. However Chapter 3 was observational in approach and its conclusions would benefit from an experimental approach. In addition, Chapter 3 only examined adult hourglass shape, and did not examine coloration during other instars or dorsal coloration. To better examine how relatedness and long term condition influence all aspects of widow coloration, black widows could be collected across the United States before rearing their young in the lab.

Young widows could be feed different diets varying in amounts and nutrition, and their coloration could be examined at all instars. In addition, the original adult widows could be genotyped to examine if genetically more similar lineages show similar coloration at different instars.

Appendix A: Additional Figures for Chapter Two

Table 7: Species of birds and bill lengths used for analysis. In cases where bill length measurements were reported separately by population, the closest one to the experimental site (Durham, NC) was used (see note). Values were averaged in cases where male and female values were given separately. All bill lengths used were the exposed culmen measurements from The Birds of North America Online (ed: A. Poole).

common name	scientific name	average bill length (mm)	note
Carolina chickadee	<i>Poecile carolinensis</i>	8.4	<i>P. c. carolinensis</i> values
house finch	<i>Haemorhous mexicanus</i>	8.68	average of NY population
tufted titmouse	<i>Baeolophus bicolor</i>	12.1	
white-breasted nuthatch	<i>Sitta carolinensis</i>	19	mean of reported ranges
northern cardinal	<i>Cardinalis cardinalis</i>	12.3	NJ, NC, PA population
red-headed woodpecker	<i>Melanerpes erythrocephalu</i>	27	
pileated woodpecker	<i>Dryocopus pileatus</i>	47	

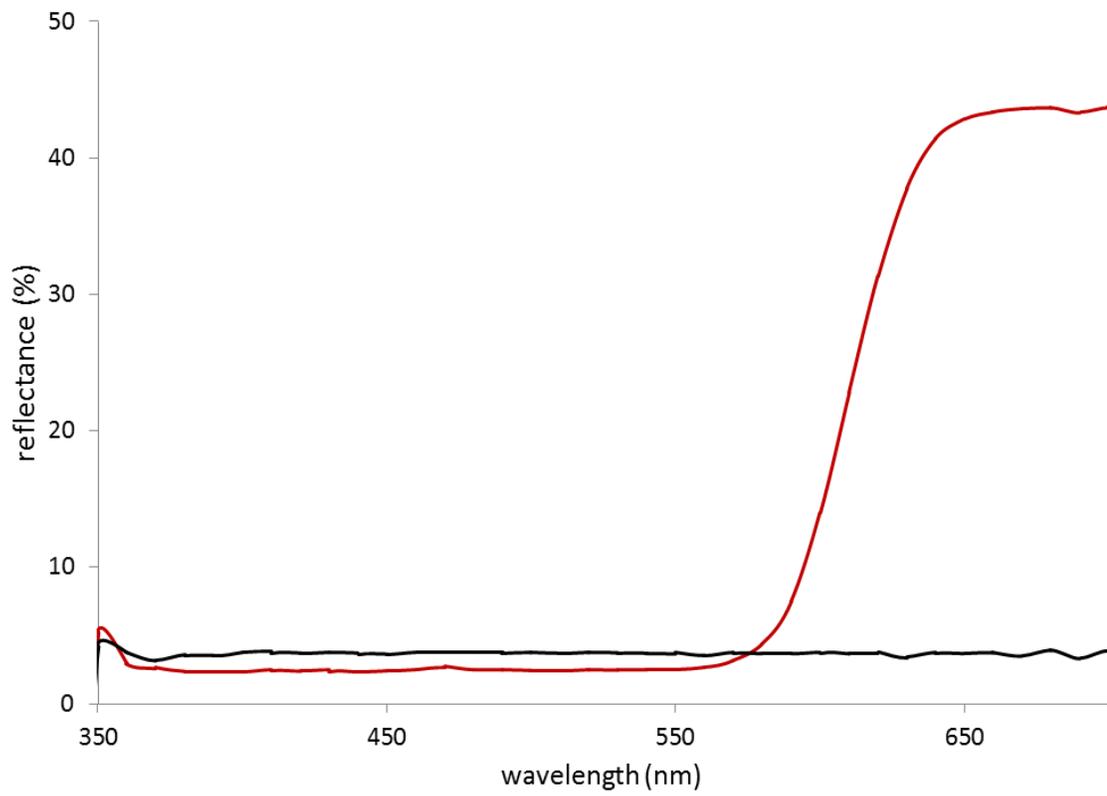


Figure 16: Reflectance of the paints used for the black widow models.

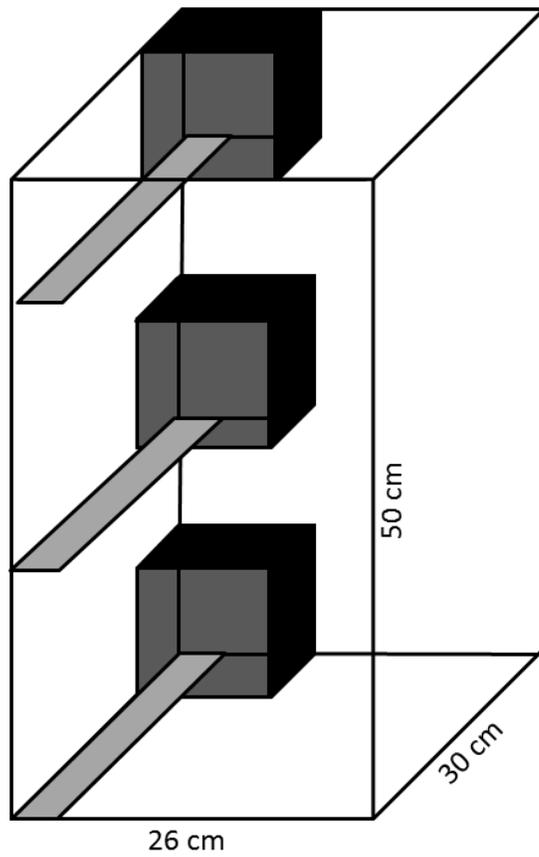


Figure 17: Schematic of the widow-towers used in the microhabitat choice experiments. View is through the wire mesh of the reptile terrarium. Scale is approximate. The three false retreats (black and dark grey cubes) were 10 cm x 10 cm x 10cm. Wooden planks to the false retreats (light grey parallelograms) were 2.5 cm wide.

Appendix B: Noise vs. Non-noise Visual Contrast Models

There are two different broad types of visual contrast models used in animal vision: non-noise limited models and noise-limited models (Kelber, Vorobyev et al. 2003). While both types of models have been used in recent work (Lind and Kelber 2011, Bybee, Yuan et al. 2012, Stevens, Lown et al. 2014), in this paper we only use a type of non-noise limited model (Euclidean color distance). As with every non-noise limited model, Euclidean color distances quantify the color difference between two regions of a visual scene when viewed by different viewers. However, non-noise limited models do not account for the signal to noise ratio involved in visual processing (Vorobyev and Osorio 1998, Warrant 2008, Warrant and Dacke 2011). Because we consider two regions to be viewed as different colors only when the signal to noise ratio is reliable enough to discriminate them, non-noise limited models cannot determine whether two stimuli are perceived as identical (or not).

Because of the limitations of non-noise limited models of color vision, many researchers have recently used noise-limited models which account for the signal to noise ratio involved in visual processing (Vorobyev 2003, Endler and Mielke 2005, Aviles, Soler et al. 2011, Stoddard and Stevens 2011). Specifically, photoreceptor noise appears to be the limiting factor of vision at threshold: as photoreceptor noise increases less optical stimuli are able to be resolved from each other (Vorobyev and Osorio 1998, Vorobyev, Brandt et al. 2001). When accounting for photoreceptor noise, a just

noticeable difference (JND) is defined as the minimal resolvable difference in color between two discrete regions by a particular viewer. Many researchers have extended the concepts of JNDs to stimuli that are well above threshold and assume that the greater the number of JNDs between stimuli the more conspicuous it is.

While we understand the drawbacks of using a non-noise limited model, we felt that the application of a noise-limited model to our study would be detrimental for a variety of reasons discussed further below.

Noise estimates for relevant species are lacking. Despite the numerous studies that use noise-limited models in avian color vision, actual behavioral or physiological measurements of photoreceptor noise in birds are lacking. For avian species that lack behavioral or physiological measurements of photoreceptor noise, photoreceptor noise has often been assumed to be proportional to the relative abundance of each photoreceptor class (Endler and Mielke 2005, Lind and Kelber 2009, Spottiswoode and Stevens 2010). However behavioral data that support the above noise-limited model in birds comes from only three species [*Columba livia*; *Leiothrix lutea*; *Melopsittacus undulates* (Vorobyev and Osorio 1998, Goldsmith and Butler 2003)] with measurements of two other avian species disagreeing with the models predictions [*Gallus gallus*; *Anas platyrhynchos*; (Lind and Kelber 2009)] . We feel uncomfortable using estimates based on the above model for black widow predators because 1) none of the measured values come from potential predators of black widows and 2) the above model ignores many

factors that vary between avian species --- such as spatial resolution (Harmening, Nikolay et al. 2009) and temporal resolution (Lisney, Rubene et al. 2011)--- that are expected to affect photoreceptor noise (Johnsen 2012).

Photoreceptor noise values likely vary between relevant prey and predator species. Even if we obtained estimated photoreceptor noise values for a relevant prey and potential predator species, it is not known if those values would be appropriate in other relevant species. Because estimates of photoreceptor noise are so scarce --- and actual measurements of photoreceptor noise even scarcer--- we do not know how photoreceptor noise measurements might change even between closely related species. For black widows this is especially relevant for estimates of prey vision. Black widows are generalist predators that fed on insects with a variety of different visual capabilities (Kaston 1970, Hodar and Sanchez-Pinero 2002, Salomon 2011). As a result, photoreceptor noise values likely would vary based on prey life style; for example photoreceptor noise is expected to change with body temperature (Aho, Donner et al. 1993), eye size (Land and Nilsson 2012), protein structure (Ala-Laurila, Donner et al. 2004, Luo, Yue et al. 2011), and integration time that varies greatly between diurnal and nocturnal species (Kelber, Balkenius et al. 2002, Roth and Kelber 2004). Because a single population of black widows has been shown to feed on eight different orders of arthropods (Salomon 2011), we do not feel the photoreceptor noise value of just one prey species will improve our estimates of visual performance.

Many optical factors that decrease the JNDs of the black widow's hourglass will also decrease the JNDs of the visual background. Because the contrast of the black widow's red hourglass against the black of the spider is likely above threshold values for most observers (Figure 3), the absolute JND values calculated for both avian predators and insect prey will be irrelevant by themselves. Rather, the behavioral consequences of the black widow's JND value will also depend on the JNDs of other elements in the visual scene (in addition to patterning and other factors). As black widows live worldwide (Garb, Gonzalez et al. 2004), we would need estimates of the background levels of JNDs in a variety of habitats to accurately reflect the behavioral relevance of the black widow's own appearance. While non-noise limited models of vision should also account for the visual background, using noise-limited models adds additional factors (besides for color sensitivity) that may make a viewer have a noisy view of a black widow will also make the visual background noisier as a result. Therefore without measurements of multiple background scenes for comparison, using JND values above threshold is inappropriate.

Photoreceptor noise-limited models are not applicable at low light levels. While we are lacking behavioral data on when black widows may interact with both their predators and prey, it is likely that at least some of the interactions between black widows and avian predators occur during the dawn and dusk hours when light levels may be low. Noise limited-models assume high light levels, and have been shown to not accurately estimate visual performance at low light levels (Vorobyev and Osorio 1998).

Appendix C: Additional Figures for Chapter Three

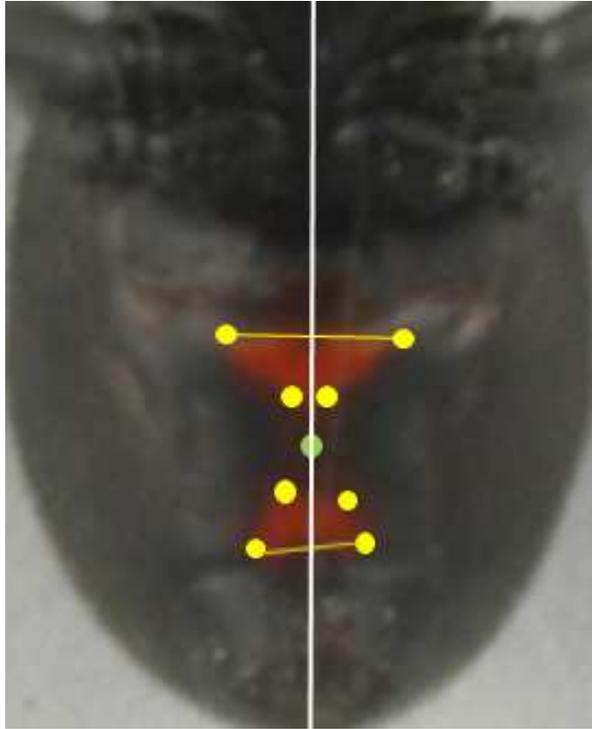


Figure 18: Hourglass modeling methods. Yellow markers: points corresponding to the corners of the upper and lower hourglass sections. Yellow lines: the sum of the slop of these two lines was set to 0 to rotate the hourglass so all were aligned in the same direction. Green marker: the centroid of all yellow markers from which distances were normalized. White line: line of bilateral symmetry. The absolute values of analogous points on either side of the white line were average to avoid issues of correlation during further statistical tests.

Table 8: Study sites and locations

County	Latitude (°N)	Longitude (°W)	Species	Geographical Region
Placer County, CA	38.8	121	<i>L. hesperus</i>	western
Ventura County, CA	34.2	119	<i>L. hesperus</i>	western
Bernalillo County, NM	35.2	107	<i>L. hesperus</i>	southwestern
El Paso County, TX	31.8	106	<i>L. hesperus</i>	southwestern
Chaves County, NM	33.6	105	<i>L. mactans</i>	southwestern
Camden County, MO	37.9	93	<i>L. variolus</i>	eastern
Clarke County, AL	31.9	88	<i>L. mactans</i>	eastern
Citrus County, FL	29.0	83	<i>L. mactans</i>	eastern
Jefferson County, KY	38.2	85	<i>L. variolus</i>	eastern

Appendix D: Additional Figures for Chapter Four

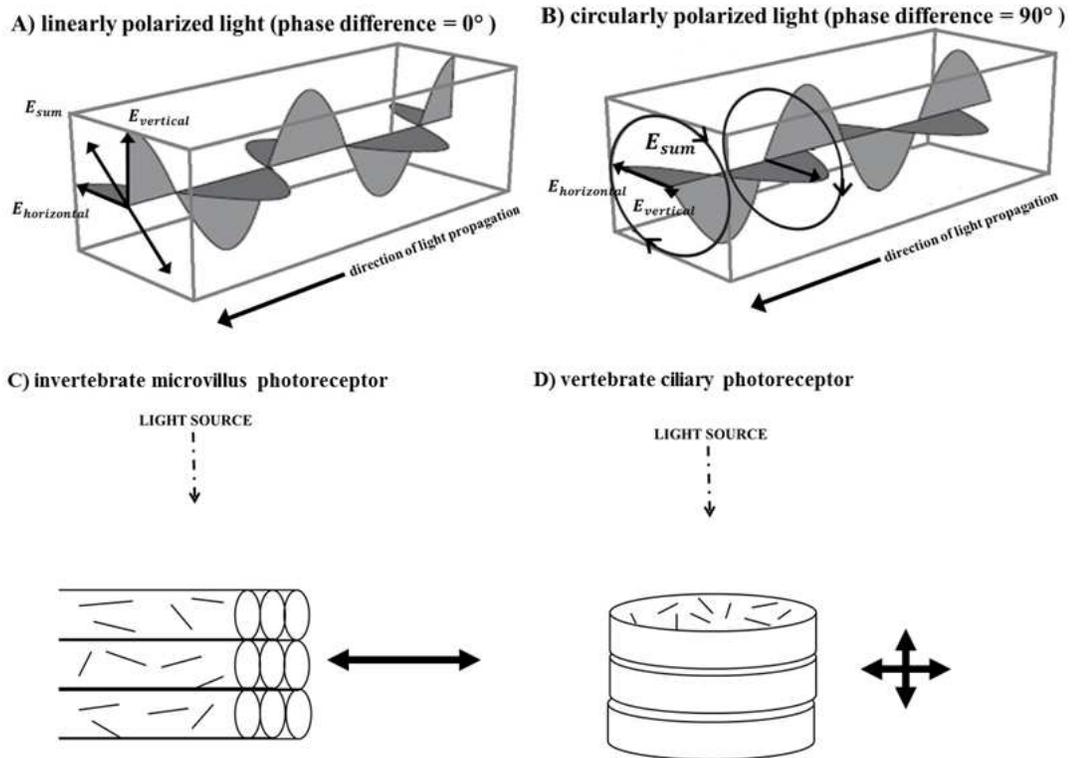


Figure 19: The polarization of light. In C and D, the unlabeled block arrows represent the polarizations each photoreceptor absorbs. A) Linearly polarized light has an e-vector along one particular axis. B) In circularly polarized light, the e-vector rotates as the light propagates. C) The photopigments expressed by the rhabdomeric photoreceptors of invertebrates are found in many elongated tubes, known as microvilli. Although the photopigments may arrange themselves randomly within the microvilli, when viewed in the plane perpendicular to the incident light, the photopigments appear to be preferentially oriented parallel to the long axes of the microvilli. As a result, the photoreceptors are more sensitive to a particular polarization plane than to any other. D) The typical ciliary photoreceptors found in vertebrates are polarization-insensitive. They resemble discs and do not bias the arrangement of photopigments in any particular direction. Therefore typical vertebrate photoreceptors respond equally to all polarizations (shown by the block arrows). A) and B) adapted from Johnsen (2012)

Appendix E: Color and Polarization of *H. cydno*'s Wing

Comparisons between the blue and white areas of the *H. cydno*'s wing show that the polarized reflections are accompanied by large color and luminance differences. Reflectance measurements of both the blue and white regions were taken using a 200 μm fiber coupled with a USB2000 spectroradiometer (Ocean Optics Inc. Dunedin, FL, USA). The incident light was perpendicular to the plane of the butterfly wing and the probe was at an angle of 45° . Using model photoreceptors, both color and polarization Michelson contrast values $[(Q1 - Q2)/(Q1+Q2)]$ of adjacent wing areas were calculated. These ratios were then compared to each other to find the polarization or color distance between the regions. We set the λ_{max} of the model polarization photoreceptor at 450 nm because this value maximized the polarization contrast. Polarized photoreceptors were set at the angles maximally and minimally sensitive to the polarization. Model λ_{max} values for the dichromatic color photoreceptors were 450 nm and 530 nm. The achromatic photoreceptor was set at 450 nm. Photon catch calculations used the Stavenga template (Stavenga, Smits et al. 1993) in the light of a terrestrial forest (Endler 1993).

References

- Adler, K. and D. H. Taylor (1973). "Extraocular Perception of Polarized-Light by Orienting Salamanders." Journal of Comparative Physiology **87**(3): 203-212.
- Aho, A. C., K. Donner, C. Hyden, L. O. Larsen and T. Reuter (1988). "Low Retinal Noise in Animals with Low Body-Temperature Allows High Visual Sensitivity." Nature **334**(6180): 348-350.
- Aho, A. C., K. Donner and T. Reuter (1993). "Retinal Origins of the Temperature Effect on Absolute Visual Sensitivity in Frogs." Journal of Physiology-London **463**: 501-521.
- Ala-Laurila, P., K. Donner and A. Koskelainen (2004). "Thermal activation and photoactivation of visual pigments." Biophysical Journal **86**(6): 3653-3662.
- Ala-Laurila, P., J. Pahlberg, A. Koskelainen and K. Donner (2004). "On the relation between the photoactivation energy and the absorbance spectrum of visual pigments." Vision Research **44**(18): 2153-2158.
- Amézquita, A., L. Castro, M. Arias, M. González and C. Esquivel (2013). "Field but not lab paradigms support generalisation by predators of aposematic polymorphic prey: the *Oophaga histrionica* complex." Evolutionary Ecology **27**(4): 769-782.
- Ammermuller, J., A. Itzhaki, R. Weiler and I. Perlman (1998). "UV-sensitive input to horizontal cells in the turtle retina." European Journal of Neuroscience **10**(5): 1544-1552.
- Anderson, J. F. (1974). "Responses to Starvation in the Spiders *Lycosa Lenta* Hentz and *Filistata Hibernalis* (Hentz)." Ecology **55**(3): 576-585.
- Andersson, M. B. (1994). Sexual selection. Princeton, N.J., Princeton University Press.
- Andrade, M. C. B. (1996). "Sexual Selection for Male Sacrifice in the Australian Redback Spider." Science **271**(5245): 70-72.
- Andrade, M. C. B. (1998). "Female hunger can explain variation in cannibalistic behavior despite male sacrifice in redback spiders." Behavioral Ecology **9**(1): 33-42.
- Andrade, M. C. B. (2003). "Risky mate search and male self-sacrifice in redback spiders." Behavioral Ecology **14**(4): 531-538.
- Arikawa, K., S. Mizuno, D. G. W. Scholten, M. Kinoshita, T. Seki, J. Kitamoto and D. G. Stavenga (1999). "An ultraviolet absorbing pigment causes a narrow-band violet

receptor and a single-peaked green receptor in the eye of the butterfly *Papilio*." Vision Research **39**(1): 1-8.

Arnold, K. E., S. L. Ramsay, C. Donaldson and A. Adam (2007). "Parental prey selection affects risk-taking behaviour and spatial learning in avian offspring." Proceedings of the Royal Society B: Biological Sciences **274**(1625): 2563-2569.

Ashmore, J. F. and G. Falk (1980). "Single-Photon Signal in Rod Bipolar Cells of the Dogfish Retina." Journal of Physiology-London **300**(Mar): 151-166.

Auburn, J. S. and D. H. Taylor (1979). "Polarized-Light Perception and Orientation in Larval Bullfrogs *Rana-Catesbeiana*." Animal Behaviour **27**(Aug): 658-668.

Aviles, J. M., J. J. Soler and N. S. Hart (2011). "Sexual selection based on egg colour: physiological models and egg discrimination experiments in a cavity-nesting bird." Behavioral Ecology and Sociobiology **65**(9): 1721-1730.

Baird, T. A. (2008). "A growth cost of experimentally induced conspicuous coloration in first-year collared lizard males." Behavioral Ecology **19**(3): 589-593.

Baldwin, J. and S. Johnsen (2011). "Effects of molting on the visual acuity of the blue crab, *Callinectes sapidus*." J Exp Biol **214**(Pt 18): 3055-3061.

Balkenius, A. and A. Kelber (2004). "Colour constancy in diurnal and nocturnal hawkmoths." Journal of Experimental Biology **207**(19): 3307-3316.

Barlow, H. B. (1956). "Retinal Noise and Absolute Threshold." Journal of the Optical Society of America **46**(8): 634-639.

Baruffaldi, L. and M. C. B. Andrade (2015). "Contact pheromones mediate male preference in black widow spiders: avoidance of hungry sexual cannibals?" Animal Behaviour **102**(0): 25-32.

Bass, M. and Optical Society of America. (1995). Handbook of optics. New York, McGraw-Hill.

Bates, D., M. Maechler, B. Bolker and S. Waler (2014). lme4: Linear mixed-effects models using Eigen and S4.

Benson, W. W. (1972). "Natural-Selection for Mullerian Mimicry in *Heliconius-Erato* in Costa-Rica." Science **176**(4037): 936-&.

- Benson, W. W. (1972). "Natural Selection for Miillerian Mimicry in *Heliconius erato* in Costa Rica." Science **176**(4037): 936-939.
- Bernard, G. D. and R. Wehner (1977). "Functional Similarities between Polarization Vision and Color-Vision." Vision Research **17**(9): 1019-1028.
- Bhatnagar, R. D. S. and J. G. Rempel (1962). "The structure, function, and postembryonic development of the male and female copulatory organs of the black widow spider *Latrodectus curacaviensis* (Muller)." Can J Zool **40**: 465-510.
- Blackledge, T. A., A. P. Summers and C. Y. Hayashi (2005). "Gumfooted lines in black widow cobwebs and the mechanical properties of spider capture silk." Zoology **108**(1): 41-46.
- Blackledge, T. A. and J. M. Zevenbergen (2007). "Condition-dependent spider web architecture in the western black widow, *Latrodectus hesperus*." Animal Behaviour **73**: 855-864.
- Blest, A. D., R. C. Hardie, P. McIntyre and D. S. Williams (1981). "The Spectral Sensitivities of Identified Receptors and the Function of Retinal Tiering in the Principal Eyes of a Jumping Spider." Journal of Comparative Physiology **145**(2): 227-239.
- Bowmaker, J. K. and A. Knowles (1977). "The visual pigments and oil droplets of the chicken retina." Vision Research **17**(7): 755-764.
- Brady, P. and M. Cummings (2010). "Natural History Note Differential Response to Circularly Polarized Light by the Jewel Scarab Beetle *Chrysina gloriosa*." American Naturalist **175**(5): 614-620.
- Brandley, N. C., D. I. Speiser and S. Johnsen (2013). "Eavesdropping on visual secrets." Evolutionary Ecology **27**(6): 1045-1068.
- Briscoe, A. D. (2001). "Functional diversification of lepidopteran opsins following gene duplication." Molecular Biology and Evolution **18**(12): 2270-2279.
- Briscoe, A. D. and L. Chittka (2001). "The evolution of color vision in insects." Annual Review of Entomology **46**: 471-510.
- Brodie, E. D. (1993). "Differential Avoidance of Coral Snake Banded Patterns by Free-Ranging Avian Predators in Costa-Rica." Evolution **47**(1): 227-235.

- Brodie, E. D. and E. D. Brodie (1980). "Differential Avoidance of Mimetic Salamanders by Free-Ranging Birds." Science **208**(4440): 181-182.
- Brower, J. V. (1958). "Experimental Studies of Mimicry in Some North-American Butterflies .1. The Monarch, Danaus-Plexippus, and Viceroy, Limenitis-Archippus-Archippus." Evolution **12**(1): 32-47.
- Bruce, M. J., M. E. Herberstein and M. A. Elgar (2001). "Signalling conflict between prey and predator attraction." Journal of Evolutionary Biology **14**(5): 786-794.
- Bybee, S. M., F. R. Yuan, M. D. Ramstetter, J. Llorente-Bousquets, R. D. Reed, D. Osorio and A. D. Briscoe (2012). "UV Photoreceptors and UV-Yellow Wing Pigments in Heliconius Butterflies Allow a Color Signal to Serve both Mimicry and Intraspecific Communication." American Naturalist **179**(1): 38-51.
- Cade, W. (1975). "Acoustically Orienting Parasitoids - Fly Phonotaxis to Cricket Song." Science **190**(4221): 1312-1313.
- Caldwell, G. S. and R. W. Rubinoff (1983). "Avoidance of Venomous Sea-Snakes by Naive Herons and Egrets." Auk **100**(1): 195-198.
- Caldwell, G. S. and R. W. Rubinoff (1983). "Avoidance of Venomous Sea Snakes by Naive Herons and Egrets." The Auk **100**(1): 195-198.
- Cameron, D. A. and E. N. Pugh (1991). "Double Cones as a Basis for a New Type of Polarization Vision in Vertebrates." Nature **353**(6340): 161-164.
- Carvalho, L. S., J. A. Cowing, S. E. Wilkie, J. K. Bowmaker and D. M. Hunt (2007). "The molecular evolution of avian ultraviolet- and violet-sensitive visual pigments." Molecular Biology and Evolution **24**(8): 1843-1852.
- Chan, T., M. Lee and T. P. Sakmar (1992). "Introduction of Hydroxyl-Bearing Amino-Acids Causes Bathochromic Spectral Shifts in Rhodopsin - Amino-Acid Substitutions Responsible for Red-Green Color Pigment Spectral Tuning." Journal of Biological Chemistry **267**(14): 9478-9480.
- Chen, C., Q. S. Song, M. Proffit, J. M. Bessiere, Z. B. Li and M. Hossaert-McKey (2009). "Private channel: a single unusual compound assures specific pollinator attraction in *Ficus semicordata*." Functional Ecology **23**(5): 941-950.

- Chiou, T.-H., L. M. Mäthger, R. T. Hanlon and T. W. Cronin (2007). "Spectral and spatial properties of polarized light reflections from the arms of squid (*Loligo pealeii*) and cuttlefish (*Sepia officinalis* L.)." The Journal of Experimental Biology **210**(20): 3624-3635.
- Chiou, T. H., S. Kleinlogel, T. Cronin, R. Caldwell, B. Loeffler, A. Siddiqi, A. Goldizen and J. Marshall (2008). "Circular polarization vision in a stomatopod crustacean." Current Biology **18**(6): 429-434.
- Chouteau, M. and B. Angers (2011). "The Role of Predators in Maintaining the Geographic Organization of Aposematic Signals." American Naturalist **178**(6): 810-817.
- Clark, D. L., A. J. Roberts and G. W. Uetz (2012). "Eavesdropping and signal matching in visual courtship displays of spiders." Biology Letters.
- Clark, R. F., S. Wetherknecker, M. V. Vance and R. Gerkin (1992). "Clinical Presentation and Treatment of Black-Widow Spider Envenomation - a Review of 163 Cases." Annals of Emergency Medicine **21**(7): 782-787.
- Clarke, G. L. (1936). "On the depth at which fish can see." Ecology **17**(3): 452-456.
- Collier, R. J., W. R. Waldron and S. Zigman (1989). "Temporal Sequence of Changes to the Gray Squirrel Retina after near-Uv Exposure." Investigative Ophthalmology & Visual Science **30**(4): 631-637.
- Cott, H. B. (1940). Adaptive coloration in animals. London,, Methuen & Co.
- Cronin, T. W., N. Shashar, R. L. Caldwell, N. J. Marshall, A. G. Cheroske and T. H. Chiou (2003). "Polarization Vision and its Role in Biological Signaling." Integrative and Comparative Biology **43**: 549-558.
- Crothers, L., E. Gering and M. Cummings (2011). "Aposematic Signal Variation Predicts Male-Male Interactions in a Polymorphic Poison Frog." Evolution **65**(2): 599-605.
- Crothers, L. R., E. J. Gering and M. E. Cummings (2012). "Is brighter better? Aposematic signal variation predicts male-male interactions in a polymorphic poison dart frog." Integrative and Comparative Biology **52**: E38-E38.
- Cummings, M. E., J. M. Jordao, T. W. Cronin and R. F. Oliveira (2008). "Visual ecology of the fiddler crab, *Uca tangeri*: effects of sex, viewer and background on conspicuousness." Animal Behaviour **75**: 175-188.

Cummings, M. E., G. G. Rosenthal and M. J. Ryan (2003). "A private ultraviolet channel in visual communication." Proceedings of the Royal Society of London Series B-Biological Sciences **270**(1518): 897-904.

D'Amour, F. E., F. E. Becker and W. v. Riper (1936). "The Black Widow Spider." The Quarterly Review of Biology **11**(2): 123-160.

Darst, C. R., M. E. Cummings and D. C. Cannatella (2006). "A mechanism for diversity in warning signals: Conspicuousness versus toxicity in poison frogs." Proceedings of the National Academy of Sciences of the United States of America **103**(15): 5852-5857.

Darwin, C. (1871). The descent of man, and selection in relation to sex. New York,, D. Appleton and company.

De Mora, S. J., S. Demers and M. Vernet (2000). The effects of UV radiation in the marine environment. Cambridge ; New York, Cambridge University Press.

De Vries, H. (1943). "The quantum character of light and its bearing upon threshold of vision, the differential sensitivity and visual acuity of the eye." Physica **10**: 553-564.

Denton, E. J., J. B. Gilpinbr and P. G. Wright (1970). "On Filters in Photophores of Mesopelagic Fish and on a Fish Emitting Red Light and Especially Sensitive to Red Light." Journal of Physiology-London **208**(2): P72-&.

Deutschlander, M. E. and J. B. Phillips (1995). "Characterization of an Ultraviolet Photoreception Mechanism in the Retina of an Amphibian, the Axolotl (Ambystoma-Mexicanum)." Neuroscience Letters **197**(2): 93-96.

Devoe, R. D. (1975). "Ultraviolet and Green Receptors in Principal Eyes of Jumping Spiders." Journal of General Physiology **66**(2): 193-207.

Douglas, J. M., T. W. Cronin, T. H. Chiou and N. J. Dominy (2007). "Light habitats and the role of polarized iridescence in the sensory ecology of neotropical nymphalid butterflies (Lepidoptera : Nymphalidae)." Journal of Experimental Biology **210**(5): 788-799.

Douglas, R. H. and N. J. Marshall (1999). A review of vertebrate and invertebrate optical filters. Adaptive Mechanisms in the Ecology of Vision. S. N. Archer, M. B. A. Djamgoz, E. R. Loew, J. C. Partridge and S. Vallerga. Dordrecht, Kluwer Academic Publishers.

- Dulai, K. S., M. von Dornum, J. D. Mollon and D. M. Hunt (1999). "The evolution of trichromatic color vision by opsin gene duplication in New World and Old World primates." Genome Research **9**(7): 629-638.
- Edgar, R. C. (2004). "MUSCLE: multiple sequence alignment with high accuracy and high throughput." Nucleic Acids Research **32**(5): 1792-1797.
- Eisner, T., I. T. Baldwin and J. Conner (1993). "Circumvention of prey defense by a predator: ant lion vs. ant." Proceedings of the National Academy of Sciences **90**(14): 6716-6720.
- Eisner, T. and M. Eisner (2000). "Defensive use of a fecal thatch by a beetle larva (*Hemisphaerota cyanea*)." Proceedings of the National Academy of Sciences of the United States of America **97**(6): 2632-2636.
- Eisner, T., M. Eisner, A. B. Attygalle, M. Deyrup and J. Meinwald (1998). "Rendering the inedible edible: Circumvention of a millipede's chemical defense by a predaceous beetle larva (*Phengodidae*)." Proceedings of the National Academy of Sciences **95**(3): 1108-1113.
- Elias, D. O., N. Lee, E. A. Hebets and A. C. Mason (2006). "Seismic signal production in a wolf spider: parallel versus serial multi-component signals." Journal of Experimental Biology **209**(6): 1074-1084.
- Elias, D. O., A. C. Mason, W. P. Maddison and R. R. Hoy (2003). "Seismic signals in a courting male jumping spider (*Araneae: Salticidae*)." J Exp Biol **206**(Pt 22): 4029-4039.
- Endler, J. (1978). A Predator's View of Animal Color Patterns. Evolutionary Biology. M. Hecht, W. Steere and B. Wallace, Springer US. **11**: 319-364.
- Endler, J. A. (1980). "Natural-Selection on Color Patterns in *Poecilia-Reticulata*." Evolution **34**(1): 76-91.
- Endler, J. A. (1983). "Natural and Sexual Selection on Color Patterns in Poeciliid Fishes." Environmental Biology of Fishes **9**(2): 173-190.
- Endler, J. A. (1991). "Variation in the Appearance of Guppy Color Patterns to Guppies and Their Predators under Different Visual Conditions." Vision Research **31**(3): 587-608.
- Endler, J. A. (1993). "The Color of Light in Forests and Its Implications." Ecological Monographs **63**(1): 1-27.

- Endler, J. A. and J. J. D. Greenwood (1988). "Frequency-Dependent Predation, Crypsis and Aposematic Coloration [and Discussion]." Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences **319**(1196): 505-523.
- Endler, J. A. and J. Mappes (2004). "Predator mixes and the conspicuousness of aposematic signals." American Naturalist **163**(4): 532-547.
- Endler, J. A. and P. W. Mielke (2005). "Comparing entire colour patterns as birds see them." Biological Journal of the Linnean Society **86**(4): 405-431.
- Fineran, B. A. and J. A. Nicol (1976). "Novel cones in the retina of the anchovy (Anchoa)." J Ultrastruct Res **54**(2): 296-303.
- Flamarique, I. N. and F. I. Harosi (2002). "Visual pigments and dichroism of anchovy cones: A model system for polarization detection." Visual Neuroscience **19**(4): 467-473.
- Flamarique, I. N., C. W. Hawryshyn and F. I. Harosi (1998). "Double-cone internal reflection as a basis for polarization detection in fish." Journal of the Optical Society of America a-Optics Image Science and Vision **15**(2): 349-358.
- Florey, E. (1969). "Ultrastructure and Function of Cephalopod Chromatophores." American Zoologist **9**(2): 429-&.
- Foelix, R. F. (2011). Biology of spiders. Oxford ; New York, Oxford University Press.
- Frank, T. M. and J. F. Case (1988). "Visual Spectral Sensitivities of Bioluminescent Deep-Sea Crustaceans." Biological Bulletin **175**(2): 261-273.
- Frank, T. M., S. Johnsen and T. Cronin (2012). "Light and vision in the deep-sea benthos: II. Vision in deep-sea crustaceans." Journal of Experimental Biology **215**(19): 3344-3353.
- Frentiu, F. D., G. D. Bernard, C. I. Cuevas, M. P. Sison-Mangus, K. L. Prudic and A. D. Briscoe (2007). "Adaptive evolution of color vision as seen through the eyes of butterflies." Proceedings of the National Academy of Sciences of the United States of America **104**: 8634-8640.
- Frentiu, F. D. and A. D. Briscoe (2008). "A butterfly eye's view of birds." Bioessays **30**(11-12): 1151-1162.
- Fritz, G., A. S. Rand and C. W. Depamphilis (1981). "The Aposematically Colored Frog, *Dendrobates-Pumilio*, Is Distasteful to the Large, Predatory Ant, *Paraponera-Clavata*." Biotropica **13**(2): 158-159.

- Gamberale-Stille, G. and B. S. Tullberg (1999). "Experienced chicks show biased avoidance of stronger signals: an experiment with natural colour variation in live aposematic prey." Evolutionary Ecology **13**(6): 579-589.
- Garb, J. E., A. Gonzalez and R. G. Gillespie (2004). "The black widow spider genus *Latrodectus* (Araneae : Theridiidae): phylogeny, biogeography, and invasion history." Molecular Phylogenetics and Evolution **31**(3): 1127-1142.
- Gittleman, J. L. and P. H. Harvey (1980). "Why Are Distasteful Prey Not Cryptic." Nature **286**(5769): 149-150.
- Glantz, R. M. (2001). "Polarization analysis in the crayfish visual system." Journal of Experimental Biology **204**(14): 2383-2390.
- Goldsmith, T. H. and B. K. Butler (2003). "The roles of receptor noise and cone oil droplets in the photopic spectral sensitivity of the budgerigar, *Melopsittacus undulatus*." Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology **189**(2): 135-142.
- Goodman, K. R., J. P. Kelley, S. C. Welter, G. K. Roderick and D. O. Elias (2015). "Rapid diversification of sexual signals in Hawaiian *Nesosydne* planthoppers (Hemiptera: Delphacidae): the relative role of neutral and selective forces." Journal of Evolutionary Biology: n/a-n/a.
- Govardovskii, V. I., N. Fyhrquist, T. Reuter, D. G. Kuzmin and K. Donner (2000). "In search of the visual pigment template." Visual Neuroscience **17**(4): 509-528.
- Grether, G. F. and R. M. Grey (1996). "Novel cost of a sexually selected trait in the rubyspot damselfly *Hetaerina americana*: Conspicuousness to prey." Behavioral Ecology **7**(4): 465-473.
- Grober, M. S. (1988). "Brittle-Star Bioluminescence Functions as an Aposematic Signal to Deter Crustacean Predators." Animal Behaviour **36**: 493-501.
- Guilford, T. and P. H. Harvey (1998). "Ornithology - The purple patch." Nature **392**(6679): 867-+.
- Gumm, J. M., E. R. Loew and T. C. Mendelson (2012). "Differences in spectral sensitivity within and among species of darters (genus *Etheostoma*)." Vision Research **55**: 19-23.
- Ham, W. T., H. A. Mueller and D. H. Sliney (1976). "Retinal Sensitivity to Damage from Short Wavelength Light." Nature **260**(5547): 153-155.

Haney, R. A., N. A. Ayoub, T. H. Clarke, C. Y. Hayashi and J. E. Garb (2014). "Dramatic expansion of the black widow toxin arsenal uncovered by multi-tissue transcriptomics and venom proteomics." BMC Genomics **15**: 366.

Harmening, W. M., P. Nikolay, J. Orłowski and H. Wagner (2009). "Spatial contrast sensitivity and grating acuity of barn owls." Journal of Vision **9**(7).

Hart, N. S. (2001). "Variations in cone photoreceptor abundance and the visual ecology of birds." Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology **187**(9): 685-697.

Hart, N. S. and M. Vorobyev (2005). "Modelling oil droplet absorption spectra and spectral sensitivities of bird cone photoreceptors." Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology **191**(4): 381-392.

Hasegawa, E. I., K. Sawada, K. Abe, K. Watanabe, K. Uchikawa, Y. Okazaki, M. Toyama and R. H. Douglas (2008). "The visual pigments of a deep-sea myctophid fish *Myctophum nitidulum* Garman; an HPLC and spectroscopic description of a non-paired rhodopsin–porphyropsin system." Journal of Fish Biology **72**(4): 937-945.

Hastad, O., J. Victorsson and A. Odeen (2005). "Differences in color vision make passerines less conspicuous in the eyes of their predators." Proceedings of the National Academy of Sciences of the United States of America **102**(18): 6391-6394.

Hinton, H. E. (1976). "Possible Significance of Red Patches of Female Crab-Spider, *Misumena-Vatia*." Journal of Zoology **180**(Sep): 35-39.

Hódar, J. and F. Sánchez-Piñero (2002). "Feeding habits of the blackwidow spider *Latrodectus lilianae* (Araneae: Theridiidae) in an arid zone of south-east Spain." Journal of Zoology **257**(01): 101-109.

Hodar, J. A. and F. Sanchez-Pinero (2002). "Feeding habits of the blackwidow spider *Latrodectus lilianae* (Araneae : Theridiidae) in an arid zone of south-east Spain." Journal of Zoology **257**: 101-109.

Holloway, G., F. Gilbert and A. Brandt (2002). "The relationship between mimetic imperfection and phenotypic variation in insect colour patterns." Proceedings of the Royal Society B: Biological Sciences **269**(1489): 411-416.

Hopkins, C. and W. Heiligenberg (1978). "Evolutionary designs for electric signals and electroreceptors in gymnotoid fishes of Surinam." Behavioral Ecology and Sociobiology **3**(2): 113-134.

Horváth, G. and D. Varjú (2004). Polarized light in animal vision : polarization patterns in nature. Berlin ; New York, Springer.

Jacobs, G. H. (1984). "Within-Species Variations in Visual Capacity among Squirrel-Monkeys (*Saimiri-Sciureus*) - Color-Vision." Vision Research **24**(10): 1267-1277.

Jacobs, G. H., J. Neitz and J. F. Deegan (1991). "Retinal Receptors in Rodents Maximally Sensitive to Ultraviolet-Light." Nature **353**(6345): 655-656.

Jansson, C. and A. von Brömssen (1981). "Winter decline of spiders and insects in spruce *Picea abies* and its relation to predation by birds." Ecography **4**(2): 82-93.

Johnsen, S. (1994). "Extraocular sensitivity to polarized light in an echinoderm." Journal of Experimental Biology **195**: 281-291.

Johnsen, S. (2002). "Cryptic and conspicuous coloration in the pelagic environment." Proceedings of the Royal Society of London Series B-Biological Sciences **269**(1488): 243-256.

Johnsen, S. (2007). "Does new technology inspire new directions? Examples drawn from pelagic visual ecology." Integrative and Comparative Biology **47**(6): 799-807.

Johnsen, S. n. (2012). The optics of life : a biologist's guide to light in nature. Princeton, NJ, Princeton University Press.

Johnsen, S. n. (2012). The optics of life : a biologist's guide to light in nature. Princeton, NJ, Princeton University Press.

Johnson, A., O. Revis and J. C. Johnson (2011). "Chemical prey cues influence the urban microhabitat preferences of Western black widow spiders, *Latrodectus hesperus*." Journal of Arachnology **39**(3): 449-453.

Johnson, J. C., P. Trubl, V. Blackmore and L. Miles (2011). "Male black widows court well-fed females more than starved females: silken cues indicate sexual cannibalism risk." Animal Behaviour **82**(2): 383-390.

Jones, F. M. (1932). "Insect coloration and the relative acceptability of insects to birds." Transactions of the Royal Entomological Society of London **80**(2): 345-371.

Joron, M. and J. L. Mallet (1998). "Diversity in mimicry: paradox or paradigm?" Trends Ecol Evol **13**(11): 461-466.

- Kamermans, M. and C. Hawryshyn (2011). "Teleost polarization vision: how it might work and what it might be good for." Philosophical Transactions of the Royal Society B-Biological Sciences **366**(1565): 742-756.
- Kapan, D. D. (2001). "Three-butterfly system provides a field test of mullerian mimicry." Nature **409**(6818): 338-340.
- Kaston, B. J. (1970). "Comparative biology of American black widow spiders." Transactions of the San Diego Society of Natural History **16**: 33-82.
- Kasumovic, M. M. and M. C. B. Andrade (2004). "Discrimination of airborne pheromones by mate-searching male western black widow spiders (*Latrodectus hesperus*): species- and population-specific responses." Canadian Journal of Zoology **82**(7): 1027-1034.
- Kats, L. B. and L. M. Dill (1998). "The scent of death: Chemosensory assessment of predation risk by prey animals." Ecoscience **5**(3): 361-394.
- Kelber, A., A. Balkenius and E. J. Warrant (2002). "Scotopic colour vision in nocturnal hawkmoths." Nature **419**(6910): 922-925.
- Kelber, A., M. Vorobyev and D. Osorio (2003). "Animal colour vision - behavioural tests and physiological concepts." Biological Reviews **78**(1): 81-118.
- Kim, J. W., G. E. Brown, I. J. Dolinsek, N. N. Brodeur, A. O. H. C. Leduc and J. W. A. Grant (2009). "Combined effects of chemical and visual information in eliciting antipredator behaviour in juvenile Atlantic salmon *Salmo salar*." Journal of Fish Biology **74**(6): 1280-1290.
- Kleinlogel, S., N. J. Marshall, J. M. Horwood and M. F. Land (2003). "Neuroarchitecture of the color and polarization vision system of the stomatopod haptosquilla." Journal of Comparative Neurology **467**(3): 326-342.
- Kreithen, M. and W. Keeton (1974). "Detection of polarized light by the homing pigeon, *Columba livia*." Journal of Comparative Physiology **89**(1): 83-92.
- Kroger, R. H. H., M. C. W. Campbell, R. D. Fernald and H. J. Wagner (1999). "Multifocal lenses compensate for chromatic defocus in vertebrate eyes." Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology **184**(4): 361-369.

- Kroger, R. H. H. and A. Gislen (2004). "Compensation for longitudinal chromatic aberration in the eye of the firefly squid, *Watasenia scintillans*." *Vision Research* **44**(18): 2129-2134.
- Land, M. F. (1972). "The physics and biology of animal reflectors." *Progress in Biophysics and Molecular Biology* **24**: 75-106.
- Land, M. F. (1997). "VISUAL ACUITY IN INSECTS." *Annual Review of Entomology* **42**(1): 147-177.
- Land, M. F. and D.-E. Nilsson (2002). *Animal eyes*. New York, Oxford University Press.
- Land, M. F. and D. E. Nilsson (2012). "Animal Eyes, Second Edition." *Animal Eyes, Second Edition*: 1-271.
- Lariviere, S. and F. Messier (1996). "Aposematic behaviour in the striped skunk, *Mephitis mephitis*." *Ethology* **102**(12): 986-992.
- Laughlin, S. B. (2001). "Energy as a constraint on the coding and processing of sensory information." *Current Opinion in Neurobiology* **11**(4): 475-480.
- Laughlin, S. B., R. R. D. van Steveninck and J. C. Anderson (1998). "A fundamental measure of the metabolic cost of neural information in the retina of the blowfly, *Calliphora vicina*." *Journal of Physiology-London* **506P**: 92p-92p.
- Leech, D. and S. Johnsen (2009). Light, photoreceptors, and UV vision. *Encyclopedia of Inland Waters*. G. Likens. **2**: 671-681.
- Levi, H. W. (1959). "The Spider Genus *Latrodectus* (Araneae, Theridiidae)." *Transactions of the American Microscopical Society* **78**(1): 7-43.
- Levine, J. S., P. S. Lobel and M. E. F. (1980). *Visual Communication in Fishes. Environmental Physiology of Fishes*. M. A. Ali. New York, Plenum Press: 447--475.
- Lewis, E. E. and J. H. Cane (1990). "Pheromonal Specificity of Southeastern *Ips* Pine Bark Beetles Reflects Phylogenetic Divergence (Coleoptera, Scolytidae)." *Canadian Entomologist* **122**(11-12): 1235-1238.
- Lind, O. and A. Kelber (2009). "Avian colour vision: Effects of variation in receptor sensitivity and noise data on model predictions as compared to behavioural results." *Vision Research* **49**(15): 1939-1947.

- Lind, O. and A. Kelber (2011). "The spatial tuning of achromatic and chromatic vision in budgerigars." Journal of Vision **11**(7).
- Lindstedt, C., H. Eager, E. Ihalainen, A. Kahilainen, M. Stevens and J. Mappes (2011). "Direction and strength of selection by predators for the color of the aposematic wood tiger moth." Behavioral Ecology.
- Lindström, L., R. V. Alatalo, A. Lyytinen, J. Mappes and M. Björklund (2004). "THE EFFECT OF ALTERNATIVE PREY ON THE DYNAMICS OF IMPERFECT BATESIAN AND MÜLLERIAN MIMICRIES." Evolution **58**(6): 1294-1302.
- Lisney, T. J., D. Rubene, J. Rozsa, H. Lovlie, O. Hastad and A. Odeen (2011). "Behavioural assessment of flicker fusion frequency in chicken *Gallus gallus domesticus*." Vision Research **51**(12): 1324-1332.
- Lloyd, J. E. and S. R. Wing (1983). "Nocturnal Aerial Predation of Fireflies by Light-Seeking Fireflies." Science **222**(4624): 634-635.
- Loew, E. R., L. J. Fleishman, R. G. Foster and I. Provencio (2002). "Visual pigments and oil droplets in diurnal lizards: a comparative study of Caribbean anoles." Journal of Experimental Biology **205**(7): 927-938.
- Loew, E. R. and J. N. Lythgoe (1978). "Ecology of Cone Pigments in Teleost Fishes." Vision Research **18**(6): 715-722.
- Losey, G. S., T. W. Cronin, T. H. Goldsmith, D. Hyde, N. J. Marshall and W. N. McFarland (1999). "The UV visual world of fishes: a review." Journal of Fish Biology **54**(5): 921-943.
- Luo, D. G., W. W. S. Yue, P. Ala-Laurila and K. W. Yau (2011). "Activation of Visual Pigments by Light and Heat." Science **332**(6035): 1307-1312.
- Lythgoe, J. N. (1968). "Visual Pigments and Visual Range Underwater." Vision Research **8**(8): 997-&.
- Lythgoe, J. N. and J. C. Partridge (1991). "The Modeling of Optimal Visual Pigments of Dichromatic Teleosts in Green Coastal Waters." Vision Research **31**(3): 361-371.
- Maan, M. E. and M. E. Cummings (2008). "FEMALE PREFERENCES FOR APOSEMATIC SIGNAL COMPONENTS IN A POLYMORPHIC POISON FROG." Evolution **62**(9): 2334-2345.

- Maan, M. E. and M. E. Cummings (2009). "Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog." Proceedings of the National Academy of Sciences of the United States of America **106**(45): 19072-19077.
- MacLeod, E. C. and M. C. B. Andrade (2014). "Strong, convergent male mate choice along two preference axes in field populations of black widow spiders." Animal Behaviour **89**(0): 163-169.
- Mallet, J. (1993). "Speciation, Racialization, and Color Pattern Evolution in Heliconius Butterflies - Evidence from Hybrid Zones." Hybrid Zones and the Evolutionary Process: 226-260.
- Mallet, J. (1999). "Causes and consequences of a lack of coevolution in mullerian mimicry." Evolutionary Ecology **13**(7-8): 777-806.
- Mallet, J. and M. Joron (1999). "Evolution of diversity in warning color and mimicry: Polymorphisms, shifting balance, and speciation." Annual Review of Ecology and Systematics **30**: 201-233.
- Mallet, J. and M. Joron (1999). "EVOLUTION OF DIVERSITY IN WARNING COLOR AND MIMICRY: Polymorphisms, Shifting Balance, and Speciation." Annual Review of Ecology and Systematics **30**(1): 201-233.
- Mallet, J. and M. C. Singer (1987). "Individual Selection, Kin Selection, and the Shifting Balance in the Evolution of Warning Colors - the Evidence from Butterflies." Biological Journal of the Linnean Society **32**(4): 337-350.
- Maroli, M., S. Bettini and B. Panfili (1972). "Toxicity of Latrodectus-Mactans-Tredecimguttatus Venom on Frog and Birds." Toxicon **11**(2): 203-203.
- Maroli, M., S. Bettini and B. Panfili (1973). "Toxicity of Latrodectus mactans tredecimguttatus venom on frog and birds." Toxicon **11**(2): 203-206.
- Marples, N. M., D. J. Kelly and R. J. Thomas (2005). "Perspective: The evolution of warning coloration is not paradoxical." Evolution **59**(5): 933-940.
- Marshall, J., J. Kent and T. Cronin (1999). Visual adaptations in crustaceans: spectral sensitivity in diverse habitats. Adaptive mechanisms in the ecology of vision. S. N. Archer. Dordrecht ; Boston, Kluwer: 285-327.

- Marshall, N. J. (2000). "Communication and camouflage with the same 'bright' colours in reef fishes." Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **355**(1401): 1243-1248.
- Mathger, L. M. and R. T. Hanlon (2006). "Anatomical basis for camouflaged polarized light communication in squid." Biology Letters **2**(4): 494-496.
- Mathger, L. M., N. Shashar and R. T. Hanlon (2009). "Do cephalopods communicate using polarized light reflections from their skin?" Journal of Experimental Biology **212**(14): 2133-2140.
- McCrone, J. D. and H. W. Levi (1966). "Postembryological development of spiderlings from two Peruvian *Latrodectus* populations." Pysche(71): 12-27.
- McShea, D. W. and R. N. Brandon (2010). Biology's first law : the tendency for diversity and complexity to increase in evolutionary systems. Chicago ; London, University of Chicago Press.
- Menzel, R. (1979). Spectral Sensitivity and Color Vision in Invertebrates. Comparative Physiology and Evolution of Vision in Invertebrates. H. Autrum, Springer Berlin Heidelberg. **7 / 6 / 6 A**: 503-580.
- Michiels, N. K., N. Anthes, N. S. Hart, J. Herler, A. J. Meixner, F. Schleifenbaum, G. Schulte, U. E. Siebeck, D. Sprenger and M. F. Wucherer (2008). "Red fluorescence in reef fish, a novel signalling mechanism?" BMC Ecology **8**(16).
- Michinomae, M., H. Masuda, M. Seidou and Y. Kito (1994). "Structural Basis for Wavelength Discrimination in the Banked Retina of the Firefly Squid *Watasenia Scintillans*." Journal of Experimental Biology **193**: 1-12.
- Moody, M. F. and J. R. Parriss (1961). "The Discrimination of Polarized Light by Octopus - a Behavioural and Morphological Study." Zeitschrift Fur Vergleichende Physiologie **44**(3): 268-291.
- Muheim, R. (2011). "Behavioural and physiological mechanisms of polarized light sensitivity in birds." Philosophical Transactions of the Royal Society B-Biological Sciences **366**(1565): 763-771.
- Müller, F. (1879). "Ituna and Thyridia: a remarkable case of mimicry in butterflies." Trans. Entomol. Soc. Lond **1879**: 20-29.

- Munz, F. W. (1958). "The Photosensitive Retinal Pigments of Fishes from Relatively Turbid Coastal Waters." Journal of General Physiology **42**(2): 445-459.
- Nakano, R., N. Skals, T. Takanashi, A. Surlykke, T. Koike, K. Yoshida, H. Maruyama, S. Tatsuki and Y. Ishikawa (2008). "Moths produce extremely quiet ultrasonic courtship songs by rubbing specialized scales." Proc Natl Acad Sci U S A **105**(33): 11812-11817.
- Narins, P. M. (1990). "Seismic Communication in Anuran Amphibians." Bioscience **40**(4): 268-274.
- Neitz, M., J. Neitz and G. H. Jacobs (1991). "Spectral Tuning of Pigments Underlying Red-Green Color-Vision." Science **252**(5008): 971-974.
- Neville, A. C. and S. Caveney (1969). "Scarabaeid Beetle Exocuticle as an Optical Analogue of Cholesteric Liquid Crystals." Biological Reviews of the Cambridge Philosophical Society **44**(4): 531-&.
- Niven, J. E., J. C. Anderson and S. B. Laughlin (2007). "Fly photoreceptors demonstrate energy-information trade-offs in neural coding." Plos Biology **5**(4): 828-840.
- Noonan, B. P. and A. A. Comeault (2009). The role of predator selection on polymorphic aposematic poison frogs.
- Odeen, A., O. Hastad and P. Alstrom (2011). "Evolution of ultraviolet vision in the largest avian radiation - the passerines." Bmc Evolutionary Biology **11**.
- Ortolani, A. (1999). "Spots, stripes, tail tips and dark eyes: Predicting the function of carnivore colour patterns using the comparative method." Biological Journal of the Linnean Society **67**(4): 433-476.
- Pagel, M. (1994). "Detecting Correlated Evolution on Phylogenies - a General-Method for the Comparative-Analysis of Discrete Characters." Proceedings of the Royal Society B-Biological Sciences **255**(1342): 37-45.
- Pahlberg, J., M. Lindstrom, P. Ala-Laurila, N. Fyhrquist-Vanni, A. Koskelainen and K. Donner (2005). "The photoactivation energy of the visual pigment in two spectrally different populations of *Mysis relicta* (Crustacea, Mysida)." Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology **191**(9): 837-844.
- Paine, R. T. (1966). "Food Web Complexity and Species Diversity." American Naturalist **100**(910): 65-&.

- Paradis, E., J. Claude and K. Strimmer (2004). "APE: Analyses of Phylogenetics and Evolution in R language." Bioinformatics **20**(2): 289-290.
- Parri, S., R. V. Alatalo, J. Kotiaho and J. Mappes (1997). "Female choice for male drumming in the wolf spider *Hygrolycosa rubrofasciata*." Animal Behaviour **53**: 305-312.
- Parry, J. W. L., K. L. Carleton, T. Spady, A. Carboo, D. M. Hunt and J. K. Bowmaker (2005). "Mix and match color vision: Tuning spectral sensitivity by differential opsin gene expression in Lake Malawi Cichlids." Current Biology **15**(19): 1734-1739.
- Partridge, J. C. and R. H. Douglas (1995). "Far-Red Sensitivity of Dragon Fish." Nature **375**(6526): 21-22.
- Pasquet, A., R. Leborgne and Y. Lubin (1999). "Previous foraging success influences web building in the spider *Stegodyphus lineatus* (Eresidae)." Behavioral Ecology **10**(2): 115-121.
- Payne, K. B., W. R. Langbauer and E. M. Thomas (1986). "Infrasonic Calls of the Asian Elephant (*Elephas-Maximus*)." Behavioral Ecology and Sociobiology **18**(4): 297-301.
- Peake, T. M. (2005). Eavesdropping in communication networks. Animal Communication Networks. P. K. McGregor. Cambridge, Cambridge University Press.
- Peake, T. M., A. M. R. Terry, P. K. McGregor and T. Dabelsteen (2001). "Male great tits eavesdrop on simulated male-to-male vocal interactions." Proceedings of the Royal Society of London Series B-Biological Sciences **268**(1472): 1183-1187.
- Perrard, A., M. Arca, Q. Rome, F. Muller, J. Tan, S. Bista, H. Nugroho, R. Baudoin, M. Baylac, J.-F. Silvain, J. M. Carpenter and C. Villemant (2014). "Geographic Variation of Melanisation Patterns in a Hornet Species: Genetic Differences, Climatic Pressures or Aposematic Constraints?" PLoS ONE **9**(4): e94162.
- Perry, R. J. and P. A. Mcnaughton (1991). "Response Properties of Cones from the Retina of the Tiger Salamander." Journal of Physiology-London **433**: 561-587.
- Plachetzki, D. C. and T. H. Oakley (2007). "Key transitions during the evolution of animal phototransduction: novelty, "tree-thinking," co-option, and co-duplication." Integrative and Comparative Biology **47**(5): 759-769.
- Plowright, R. C. and R. E. Owen (1980). "The Evolutionary Significance of Bumble Bee Color Patterns - a Mimetic Interpretation." Evolution **34**(4): 622-637.

- Poulton, E. B. (1890). The colours of animals. New York,, D. Appleton and company.
- Puurtinen, M. and V. Kaitala (2006). "CONDITIONS FOR THE SPREAD OF CONSPICUOUS WARNING SIGNALS: A NUMERICAL MODEL WITH NOVEL INSIGHTS." Evolution **60**(11): 2246-2256.
- R Core Team (2013). R: A Language and Environment for Statistical Computing. Vienna, Austria.
- Ramsay, S. L. and D. C. Houston (2003). "Amino acid composition of some woodland arthropods and its implications for breeding tits and other passerines." Ibis **145**(2): 227-232.
- Reilly, C. R. L. and S. H. Thompson (2007). "Temperature effects on low-light vision in juvenile rockfish (Genus *Sebastes*) and consequences for habitat utilization." Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology **193**(9): 943-953.
- Roberts, N. W., M. L. Porter and T. W. Cronin (2011). "The molecular basis of mechanisms underlying polarization vision." Philosophical Transactions of the Royal Society B-Biological Sciences **366**(1565): 627-637.
- Romer, H., A. Lang and M. Hartbauer (2010). "The signaller's dilemma: a cost-benefit analysis of public and private communication." PLoS One **5**(10): e13325.
- Roper, T. J. and S. E. Cook (1989). "Responses of Chicks to Brightly Colored Insect Prey." Behaviour **110**: 276-293.
- Roper, T. J. and S. Redston (1987). "Conspicuousness of Distasteful Prey Affects the Strength and Durability of One-Trial Avoidance-Learning." Animal Behaviour **35**: 739-747.
- Ross, K. and R. L. Smith (1979). "Aspects of the Courtship Behavior of the Black Widow Spider, *Latrodectus hesperus* (Araneae: Theridiidae), with Evidence for the Existence of a Contact Sex Pheromone." Journal of Arachnology **7**(1): 69-77.
- Roth, L. S. V. and A. Kelber (2004). "Nocturnal colour vision in geckos." Proceedings of the Royal Society B-Biological Sciences **271**: S485-S487.
- Rowe, C. and T. Guilford (1999). "The evolution of multimodal warning displays." Evolutionary Ecology **13**(7-8): 655-671.

- Rowe, C., L. Lindström and A. Lyytinen (2004). "The importance of pattern similarity between Müllerian mimics in predator avoidance learning." Proceedings of the Royal Society of London. Series B: Biological Sciences **271**(1537): 407-413.
- Rowland, H. M., E. Ihalainen, L. Lindstrom, J. Mappes and M. P. Speed (2007). "Co-mimics have a mutualistic relationship despite unequal defences." Nature **448**(7149): 64-67.
- Ruxton, G. D., T. N. Sherratt and M. P. Speed (2004). Avoiding attack : the evolutionary ecology of crypsis, warning signals, and mimicry. Oxford ; New York, Oxford University Press.
- Sakura, M., K. Takasuga, M. Watanabe and E. Eguchi (2003). "Diurnal and circadian rhythm in compound eye of cricket (*Gryllus bimaculatus*): Changes in structure and photon capture efficiency." Zoological Science **20**(7): 833-840.
- Salcedo, E., L. J. Zheng, M. Phistry, E. E. Bagg and S. G. Britt (2003). "Molecular basis for ultraviolet vision in invertebrates." Journal of Neuroscience **23**(34): 10873-10878.
- Salomon, M. (2011). "The natural diet of a polyphagous predator, *Latrodectus hesperus* (Araneae: Theridiidae), over one year." Journal of Arachnology **39**(1): 154-160.
- Salomon, M. (2011). "The natural diet of a polyphagous predator, *Latrodectus hesperus* (Araneae: Theridiidae), over one year." The Journal of Arachnology **39**(1): 154+.
- SAS Insitute Inc. (2013). JMP®. Cary, NC.
- Schaefer, H. M., V. Schaefer and D. J. Levey (2004). "How plant-animal interactions signal new insights in communication." Trends in Ecology & Evolution **19**(11): 577-584.
- Schneider, C. A., W. S. Rasband and K. W. Eliceiri (2012). "NIH Image to ImageJ: 25 years of image analysis." Nat Methods **9**(7): 671-675.
- Schwarz, G. (1978). "Estimating the dimension of a model." The annals of statistics **6**(2): 461-464.
- Seago, A. E., P. Brady, J. P. Vigneron and T. D. Schultz (2009). "Gold bugs and beyond: a review of iridescence and structural colour mechanisms in beetles (Coleoptera)." Journal of the Royal Society Interface **6**: S165-S184.
- Seidou, M., M. Sugahara, H. Uchiyama, K. Hiraki, T. Hamanaka, M. Michinomae, K. Yoshihara and Y. Kito (1990). "On the 3 Visual Pigments in the Retina of the Firefly

Squid, *Watasenia-Scintillans*." Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology **166**(6): 769-773.

Shashar, N., P. S. Rutledge and T. W. Cronin (1996). "Polarization vision in cuttlefish - A concealed communication channel?" Journal of Experimental Biology **199**(9): 2077-2084.

Sherratt, T. N. (2008). "The evolution of Müllerian mimicry." Naturwissenschaften **95**(8): 681-695.

Shi, Y. S., F. B. Radlwimmer and S. Yokoyama (2001). "Molecular genetics and the evolution of ultraviolet vision in vertebrates." Proceedings of the National Academy of Sciences of the United States of America **98**(20): 11731-11736.

Shuter, B. J., N. P. Lester, J. LaRose, C. F. Purchase, K. Vascotto, G. Morgan, N. C. Collins and P. A. Abrams (2005). "Optimal life histories and food web position: linkages among somatic growth, reproductive investment, and mortality." Canadian Journal of Fisheries and Aquatic Sciences **62**(4): 738-746.

Siddiqi, A., T. W. Cronin, E. R. Loew, M. Vorobyev and K. Summers (2004). "Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*." J Exp Biol **207**(Pt 14): 2471-2485.

Siddiqi, A., T. W. Cronin, E. R. Loew, M. Vorobyev and K. Summers (2004). "Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*." Journal of Experimental Biology **207**(14): 2471-2485.

Siebeck, U. E. (2004). "Communication in coral reef fish: the role of ultraviolet colour patterns in damselfish territorial behaviour." Animal Behaviour **68**: 273-282.

Siebeck, U. E. and N. J. Marshall (2001). "Ocular media transmission of coral reef fish - can coral reef fish see ultraviolet light?" Vision Research **41**(2): 133-149.

Siebeck, U. E., A. N. Parker, D. Sprenger, L. M. Mathger and G. Wallis (2010). "A Species of Reef Fish that Uses Ultraviolet Patterns for Covert Face Recognition." Current Biology **20**(5): 407-410.

Sillentullberg, B. (1985). "Higher Survival of an Aposematic Than of a Cryptic Form of a Distasteful Bug." Oecologia **67**(3): 411-415.

Sillentullberg, B. (1985). "The Significance of Coloration Per Se, Independent of Background, for Predator Avoidance of Aposematic Prey." Animal Behaviour **33**(Nov): 1382-1384.

Smith, M. J. and D. G. C. Harper (1995). "Animal Signals: Models and Terminology." Journal of Theoretical Biology **177**(3): 305-311.

Smith, S. M. (1975). "Innate Recognition of Coral Snake Pattern by a Possible Avian Predator." Science **187**(4178): 759-760.

Spaethe, J. and A. D. Briscoe (2004). "Early duplication and functional diversification of the opsin gene family in insects." Molecular Biology and Evolution **21**(8): 1583-1594.

Speed, M. P. (2000). "Warning signals, receiver psychology and predator memory." Animal Behaviour **60**: 269-278.

Spottiswoode, C. N. and M. Stevens (2010). "Visual modeling shows that avian host parents use multiple visual cues in rejecting parasitic eggs." Proceedings of the National Academy of Sciences of the United States of America **107**(19): 8672-8676.

Stauffer, H. P. and R. D. Semlitsch (1993). "Effects of Visual, Chemical and Tactile Cues of Fish on the Behavioral-Responses of Tadpoles." Animal Behaviour **46**(2): 355-364.

Stavenga, D. G., R. P. Smits and B. J. Hoenders (1993). "Simple Exponential Functions Describing the Absorbency Bands of Visual Pigment Spectra." Vision Research **33**(8): 1011-1017.

Stevens, M. and I. C. Cuthill (2007). "Hidden messages: Are ultraviolet signals a special channel in avian communication?" Bioscience **57**(6): 501-507.

Stevens, M., A. E. Lown and L. E. Wood (2014). "Colour change and camouflage in juvenile shore crabs *Carcinus maenas*." Frontiers in Ecology and Evolution **2**.

Stevens, M. and G. D. Ruxton (2012). "Linking the evolution and form of warning coloration in nature." Proceedings of the Royal Society B-Biological Sciences **279**(1728): 417-426.

Stevens, M. and G. D. Ruxton (2012). "Linking the evolution and form of warning coloration in nature." Proc Biol Sci **279**(1728): 417-426.

Stoddard, M. C. and M. Stevens (2011). "Avian Vision and the Evolution of Egg Color Mimicry in the Common Cuckoo." Evolution **65**(7): 2004-2013.

Stoddard, P. K. (1999). "Predation enhances complexity in the evolution of electric fish signals." Nature **400**(6741): 254-256.

Stoltz, J. A. and M. C. B. Andrade (2010). Female's courtship threshold allows intruding males to mate with reduced effort.

Stuart-Fox, D. and A. Moussalli (2009). "Camouflage, communication and thermoregulation: lessons from colour changing organisms." Philosophical Transactions of the Royal Society B-Biological Sciences **364**(1516): 463-470.

Svadova, K., A. Exnerova, P. Stys, E. Landova, J. Valenta, A. Fucikova and R. Socha (2009). "Role of different colours of aposematic insects in learning, memory and generalization of naive bird predators." Animal Behaviour **77**(2): 327-336.

Svensson, G. P., T. Okamoto, A. Kawakita, R. Goto and M. Kato (2010). "Chemical ecology of obligate pollination mutualisms: testing the 'private channel' hypothesis in the *Breynia-Epiccephala* association." New Phytologist **186**(4): 995-1004.

Sweeney, A., C. Jiggins and S. Johnsen (2003). "Insect communication: Polarized light as a butterfly mating signal." Nature **423**(6935): 31-32.

Taylor, R. C., B. W. Buchanan and J. L. Doherty (2007). "Sexual selection in the squirrel treefrog *Hyla squirella*: the role of multimodal cue assessment in female choice." Animal Behaviour **74**: 1753-1763.

Turner, J. R., E. M. White, M. A. Collins, J. C. Partridge and R. H. Douglas (2009). "Vision in lanternfish (Myctophidae): Adaptations for viewing bioluminescence in the deep-sea." Deep-Sea Research Part I-Oceanographic Research Papers **56**(6): 1003-1017.

Tuttle, M. D. and M. J. Ryan (1981). "Bat Predation and the Evolution of Frog Vocalizations in the Neotropics." Science **214**(4521): 677-678.

Vamosi, S. M., K. Przeczek and C. Mueller (2008). "The evolution of aposematism is accompanied by increased diversification." Integrative Zoology **3**(3): 149-156.

Venables, W. N., B. D. Ripley and W. N. Venables (2002). Modern applied statistics with S. New York, Springer.

Vetter, R. S. (1980). "Defensive Behavior of the Black-Widow Spider *Latrodectus-Hesperus* (Araneae, Theridiidae)." Behavioral Ecology and Sociobiology **7**(3): 187-193.

Viitala, J., E. Korpimäki, P. Palokangas and M. Koivula (1995). "Attraction of Kestrels to Vole Scent Marks Visible in Ultraviolet-Light." Nature **373**(6513): 425-427.

- Vorobyev, M. (2003). "Coloured oil droplets enhance colour discrimination." Proceedings of the Royal Society B-Biological Sciences **270**(1521): 1255-1261.
- Vorobyev, M., R. Brandt, D. Peitsch, S. B. Laughlin and R. Menzel (2001). "Colour thresholds and receptor noise: behaviour and physiology compared." Vision Research **41**(5): 639-653.
- Vorobyev, M. and D. Osorio (1998). "Receptor noise as a determinant of colour thresholds." Proceedings of the Royal Society B-Biological Sciences **265**(1394): 351-358.
- Vorobyev, M. and D. Osorio (1998). "Receptor noise as a determinant of colour thresholds." Proc Biol Sci **265**(1394): 351-358.
- Wagner, W. E. (1996). "Convergent song preferences between female field crickets and acoustically orienting parasitoid flies." Behavioral Ecology **7**(3): 279-285.
- Wald, G. and S. Rayport (1977). "Vision in Annelid Worms." Science **196**(4297): 1434-1439.
- Walla, P., F. G. Barth and E. Eguchi (1996). "Spectral sensitivity of single photoreceptor cells in the eyes of the ctenid spider *Cupiennius salei* Keys." Zoological Science **13**(1): 199-202.
- Wang, I. J. (2011). "Inversely Related Aposematic Traits: Reduced Conspicuousness Evolves with Increased Toxicity in a Polymorphic Poison-Dart Frog." Evolution **65**(6): 1637-1649.
- Wang, I. J. and H. B. Shaffer (2008). "Rapid Color Evolution in an Aposematic Species: A Phylogenetic Analysis of Color Variation in the Strikingly Polymorphic Strawberry Poison-Dart Frog." Evolution **62**(11): 2742-2759.
- Warrant, E. and M. Dacke (2011). "Vision and Visual Navigation in Nocturnal Insects." Annual Review of Entomology, Vol 56 **56**: 239-254.
- Warrant, E. J. (2008). "Seeing in the dark: vision and visual behaviour in nocturnal bees and wasps." Journal of Experimental Biology **211**(11): 1737-1746.
- Warrant, E. J. (2010). "Polarisation Vision: Beetles See Circularly Polarised Light." Current Biology **20**(14): R610-R612.
- Warrant, E. J. and N. A. Locket (2004). "Vision in the deep sea." Biological Reviews **79**(3): 671-712.

- Waterman, T. H. and R. B. Forward (1970). "Field Evidence for Polarized Light Sensitivity in Fish *Zenarchopterus*." Nature **228**(5266): 85-&.
- White, E. M., D. M. Goncalves, J. C. Partridge and R. F. Oliveira (2004). "Vision and visual variation in the peacock blenny." Journal of Fish Biology **65**(1): 227-250.
- Widder, E. A., M. I. Latz, P. J. Herring and J. F. Case (1984). "Far Red Bioluminescence from 2 Deep-Sea Fishes." Science **225**(4661): 512-514.
- Wilkins, L. A. (1984). "Ultraviolet Sensitivity in Hyperpolarizing Photoreceptors of the Giant Clam *Tridacna*." Nature **309**(5967): 446-448.
- Williams, N. H. and C. H. Dodson (1972). "Selective Attraction of Male Euglossine Bees to Orchid Floral Fragrances and Its Importance in Long-Distance Pollen Flow." Evolution **26**(1): 84-&.
- Wood, A. M. and R. J. W. Truscott (1993). "Uv Filters in Human Lenses - Tryptophan Catabolism." Experimental Eye Research **56**(3): 317-325.
- Wynberg, H., E. W. Meijer, J. C. Hummelen, H. P. J. M. Dekkers, P. H. Schippers and A. D. Carlson (1980). "Circular-Polarization Observed in Bioluminescence." Nature **286**(5773): 641-642.
- Yachi, S. and M. Higashi (1998). "The evolution of warning signals." Nature **394**(6696): 882-884.
- Yamashita, S. and H. Tateda (1976). "Spectral Sensitivities of Jumping Spider Eyes." Journal of Comparative Physiology **105**(1): 29-41.
- Yokoyama, S. (1994). "Gene Duplications and Evolution of the Short Wavelength-Sensitive Visual Pigments in Vertebrates." Molecular Biology and Evolution **11**(1): 32-39.
- Yokoyama, S. (2000). "Molecular evolution of vertebrate visual pigments." Progress in Retinal and Eye Research **19**(4): 385-419.
- Yokoyama, S. and F. B. Radlwimmer (2001). "The molecular genetics and evolution of red and green color vision in vertebrates." Genetics **158**(4): 1697-1710.
- Yokoyama, S., F. B. Radlwimmer and N. S. Blow (2000). "Ultraviolet pigments in birds evolved from violet pigments by a single amino acid change." Proceedings of the National Academy of Sciences of the United States of America **97**(13): 7366-7371.
- Zahavi, A. (1975). "Mate selection-a selection for a handicap." J Theor Biol **53**(1): 205-214.

Zahavi, A. (1977). "The cost of honesty (further remarks on the handicap principle)." J Theor Biol **67**(3): 603-605.

Zeiger, J. and T. H. Goldsmith (1989). "Spectral Properties of Porphyropsin from an Invertebrate." Vision Research **29**(5): 519-527.

Zigman, S. and S. J. Bagley (1971). "Near Ultraviolet Light Effects on Dogfish Retinal Rods." Experimental Eye Research **12**(1): 155-&.

Zuk, M. and G. R. Kolluru (1998). "Exploitation of sexual signals by predators and parasitoids." Quarterly Review of Biology **73**(4): 415-438.

Zwickl, D. J. (2006). Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph. D., The University of Texas at Austin.

Zylinski, S. and D. Osorio (2013). "Visual contrast and color in rapid learning of novel patterns by chicks." Journal of Experimental Biology **216**: 4184-4289.

Biography

Nicholas Brandley was born in Berkeley, CA on December 13th 1985. He received a Bachelor of Science with honors from the University of Michigan, Ann Arbor in April, 2008. He began graduate school at Duke University in August 2009. Since that time he has published two articles including: “*A predator-elicited vocalisation in the Variegated Fairy-wren (Malurus lamberti)*” in *Emu* (2010) and “*Eavesdropping on visual secrets*” in *Evolutionary Ecology* (2013). As a graduate student, Brandley received the Anne T. and Robert M. Bass Fellowship for Undergraduate Instruction (2013-2014), a James B. Duke Fellowship (2009-2013), two Summer Research Fellowships from Duke University (2012-2013) and three Grant-in-aid-of-research from Duke Biology (2011, 2012, 2014). After graduation he plans to continue teaching biology.