



Behavioral Ecology (2016), 00(00), 1–9. doi:10.1093/beheco/arw014

## Original Article

# Aposematic signals in North American black widows are more conspicuous to predators than to prey

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Received 22 January 2015; revised 12 January 2016; accepted 23 January 2016.

The iconic red hourglass of the black widow spiders (genus *Latrodectus*) is traditionally considered an aposematic signal, yet experimental evidence is lacking. Here, we present data that suggest that black widow coloration may have evolved to be an aposematic signal that is more conspicuous to their vertebrate predators than to their insect prey. In choice experiments with wild birds, we found that the red-and-black coloration deters potential predators: Wild birds were ~3 times less likely to attack a black widow model with an hourglass than one without. Using visual-system appropriate models, we also found that a black widow's red-and-black color combo is more apparent to a typical bird than a typical insect. Additionally, an ancestral reconstruction reveals that red dorsal coloration is ancestral in black widows and that at some point some North American widows lost their red dorsal coloration. Behaviorally, differences in red dorsal coloration between 2 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 trade-off of being more conspicuous to predators than to prey. We suggest that limiting detection by prey may help explain why red and black aposematic signals occur frequently in nature.

**Key words:** aposematism, communication, *Latrodectus*, private signaling, warning signals.

## INTRODUCTION

Well-defended animals may benefit if they signal to predators that they are dangerous or otherwise distasteful (Cott 1940; Ruxton 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; Sillén-Tullberg 1985a). 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; Sillén-Tullberg 1985b; 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 et al. 2011, 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 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, selection should favor aposematic signals that are more conspicuous to potential predators than to potential prey. Differences in sensory physiology and behavior between predators and prey may make this possible. 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 et al. 2003; Mathger et al. 2009; Brandley et al. 2013). Many nonaposematic signalers may use colors at the edge of the ultraviolet (UV)–visible spectrum (e.g., UV 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 et al. 2013). Additionally, if predators and prey typically view signalers from different positions (angles or distances), signals

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could be more conspicuous to typical predator positions than to typical prey positions (Marshall 2000; Cummings et al. 2008; Lind and Kelber 2011). For example, the nonaposematic 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 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 are more conspicuous to predators than prey. Adult female black widows may have 2 different types of red markings: All species possess the iconic ventral red hourglass, whereas 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 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 et al. 1973). When black widows move along their webs, they typically present their ventral hourglass upwards (Blackledge 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 Brömsen 1981; Hódar and Sánchez-Piñero 2002; Ramsay and Houston 2003; Arnold et al. 2007), so a conspicuous aposematic signal toward 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; Hódar and Sánchez-Piñero 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 et al. 2007). If black widows use a hue of red that minimizes non-red reflectance (including reflectance in the UV), 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, whereas the web of the black widow is specifically designed to catch insects approaching from below (Blackledge 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 2 species of sympatric black widows that differ in dorsal coloration. Taken together, our



**Figure 1**

Typical dorsal (a–c) and ventral (d–f) patterning of 3 species of North American *Latrodectus*. Note that black widows typically position themselves so that the ventral hourglass faces upwards. Although all 3 species have a ventral red hourglass, only *Latrodectus variolus* typically has red dorsal spots. Although these photos represent a typical individual, ventral patterning can vary greatly between populations and individuals of the same species.

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.

## PRESENTATIONS OF BLACK WIDOW MODELS TO BIRDS

### Methods

Experiments were conducted under Duke University's Institutional Animal Care & Use Committee protocol A045-11-02. In order to determine whether the red-and-black coloration of black widows deters avian predators, we purchased a digital rendering of a 3D black widow model that closely resembled *Latrodectus mactans* (abdomen length = 0.9 cm, cephalothorax length = 0.5 cm; TurboSquid Inc., New Orleans, LA). We then used the rendering to print non-toxic black widow models using a 3D printer (vero blue; C. Ideas, Crystal Lake, IL). Each black widow model ( $n = 4$  of each type) 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 (Supplementary Figure S1; black, Heavy Body Mars Black, Liquitex Artist Materials, Piscataway, NJ; red, Berry Red, DecoArt, Stanford, KY). To attract wild birds for the experimental model presentations, 7 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 seed was removed, and a single 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) for up to 2 h. To limit the effects of the location of a site and a bird's prior experience, each site received both an all-black model and an hourglass model; however, the models were presented at least a week apart and the first model type presented altered between sites. Within a model type, the exact model presented was rotated between presentations (or during presentations if a model was damaged). 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 by an observer as either landing on the feeder without touching the black widow model (avoid) or touching the model with the beak or foot (attack). Because 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 2 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 2 different models of black widows were analyzed in R using a generalized linear model (glm in

the LME4 package). We used both the Akaike information criterion (AIC) and Bayesian information criterion (BIC) to find the most parsimonious glm. Because our initial results suggested that bird size may play a role in the tendency to attack a model black widow, we ran 3 different analyses. The first analysis looked at bird species, the second analysis replaced the bird species variable with the average beak size of each species, and the third analysis replaced bird species with a measurement of overall body size (wing span; Supplementary Table S1).

Each analysis examined the response of birds (avoid/attack) to our 2 different model types of black widows (all-black/hourglass). In addition to examining one of bird species, beak length, and wing size, each analysis also examined the possible effects of seed at the feeder prior to experimentation (Seed Type), order in which the black widow models were presented (Order), the site of experimentation (Site), and any interactions between significant predictors (Table 1).

### 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 2a;  $P < 0.01$ ; glm, estimate =  $-1.56$ , standard error [SE] =  $0.78$ ,  $z = -2.01$ ). Analyses using AIC and BIC both agreed that the most parsimonious glm 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, order of model black widow presentations, site, or interactions between variables (Table 1). Despite small sample sizes, certain species of birds differed in how aggressive they were toward both the all-black and the hourglass models. The glm indicated that house finches were likely the least aggressive bird (9% attack rate,  $n = 32$ ), whereas 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 (Supplementary Table S1), we found that beak size was a strong predictor of attack likelihood for both the red hourglass and all-black model (Figure 2b; Table 1;  $P < 0.001$ ; glm, estimate =  $-5.33$ , SE =  $1.42$ ,  $z = -3.77$ ). Although wing span was also a significant predictor of attack likelihood ( $P < 0.05$ ; glm, estimate =  $-5.24$ , SE =  $2.08$ ,  $z = -2.51$ ), the AIC and BIC for wing span models were consistently higher than those using beak size, suggesting that beak size is a more relevant parameter than overall body size (Table 1).

## REFLECTANCE MEASUREMENTS AND VISUAL MODELING OF BLACK WIDOW COLORATION

### 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 *Latrodectus variolus* using a 200  $\mu\text{m}$  fiber coupled with a USB2000 spectroradiometer (Ocean Optics Inc., Dunedin, FL) and WS-1 reflectance standard (Ocean Optics Inc.). The incident light (LS-1\_CAL calibrated light source; Ocean Optics Inc.) was perpendicular

**Table 1**  
**Summary of selected glms from the presentations of black widow models to birds experiment**

Model	df	AIC	BIC	Log-likelihood	$\chi^2$ test <sup>a</sup>	$P(> \chi^2)^a$
Species glms						
Response ~ model	2	101.3	106.2	-48.6	—	—
<b>Response ~ model + species</b>	<b>8</b>	<b>82.6</b>	<b>102.4</b>	<b>-33.3</b>	<b>30.6</b>	<b>0.00003</b>
Response ~ model + species + seed type	9	83.5	105.8	-32.7	1.06	0.30
Response ~ model + species + order	9	84.2	106.6	-33.1	0.33	0.56
Response ~ model + species + site	13	86.4	118.6	-30.2	6.16	0.29
Response ~ model × species	12	87.7	117	-31.7	3.30	0.51
Beak length glms						
Response ~ model	2	101.3	106.2	-48.6	—	—
<b>Response ~ model + beak length</b>	<b>3</b>	<b>78.6</b>	<b>86.1</b>	<b>-36.3</b>	<b>24.6</b>	<b>0.0000007</b>
Response ~ model + beak length + seed type	4	80	90.2	-36.1	0.40	0.52
Response ~ model + beak length + order	4	79.8	89.7	-35.9	0.92	0.34
Response ~ model + species + site	8	82.1	101.9	-33.0	6.60	0.25
Response ~ model × beak length	4	80.6	90.5	-36.3	0.05	0.82
Wing span glms						
Response ~ model	2	101.3	106.2	-48.6	—	—
<b>Response ~ model + wing size</b>	<b>3</b>	<b>92.5</b>	<b>99.9</b>	<b>-43.3</b>	<b>10.8</b>	<b>0.001</b>
Response ~ model + wing size + seed type	4	94.5	104.4	-43.3	0.001	0.97
Response ~ model + wing size + order	4	94.4	104.3	-43.2	0.11	0.73
Response ~ model + wing size + site	8	96.4	116.0	-40.2	6.00	0.30
Response ~ model × wing size	4	94.1	104	-43	0.41	0.52

The most parsimonious glm for each category is in bold.

<sup>a</sup>Compared with most parsimonious glm located above in the same category. See methods for full descriptions of variables.

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:

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

where  $R$  is the measured spectral reflectance of the black widows,  $I$  is the reference solar spectral irradiance (<http://rredc.nrel.gov/solar/spectra/am1.5/>), and  $S$  is the spectral sensitivity of the photoreceptor in question. For the typical spectral sensitivity of the green channel in an insect ( $\lambda_{\max} = 520$  nm), we used the Govardovskii et al. (2000) visual pigment template with the rhabdom length of a field cricket (*Gryllus bimaculatus*; Sakura et al. 2003), and for spectral sensitivity of the red channel in a bird ( $\lambda_{\max} = 607$  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 Michelson contrast ( $C$ ) of the red-and-black coloration for the longest-wavelength photoreceptor for both a bird and insect where:

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

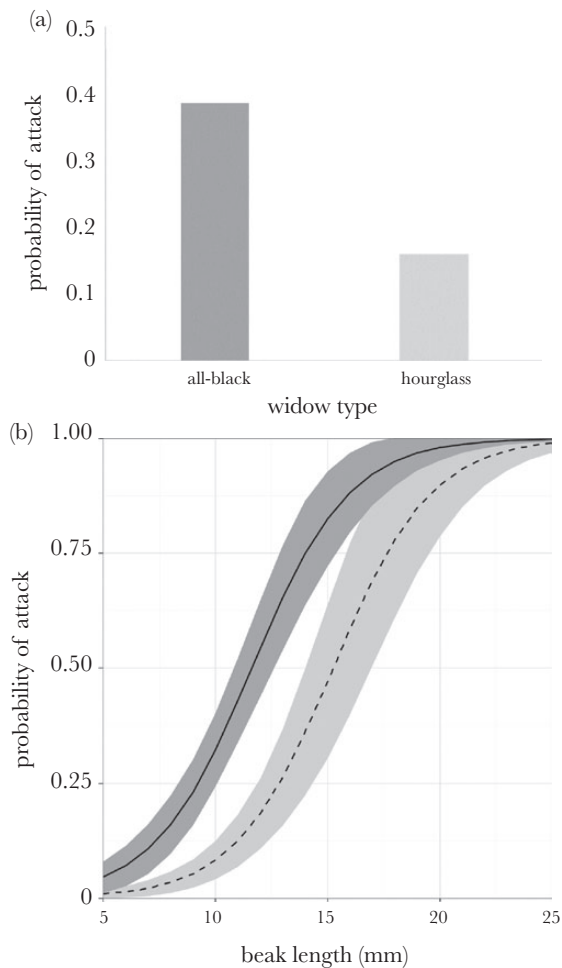
In addition to the Michelson contrast of the longest-wavelength channel, we also calculated the Euclidean color distance as a non-noise limited model of animal vision. Note that while a noise-limited model of animal vision would be ideal (Vorobyev and

Osorio 1998; Vorobyev 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 [Supplementary Material A](#) for further discussion). For insects, we calculated the Euclidean color distance of the hourglass from the black abdomen of each widow using a Maxwell triangle 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 (<http://rredc.nrel.gov/solar/spectra/am1.5/>). For the insect Maxwell triangle, we used a UV, blue, and green photoreceptor with lambda maxes at 350, 450, and 520 nm, respectively. For birds, we used tetracolorspace (Stoddard and Prum 2008) using receptor curves of the blue tit reported in Hart and Vorobyev (2005).

## Results

The red hourglass markings from adult female black widows have minimal reflectance below 550 nm (Figure 3a). Notably, adult female black widows have no significant UV 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 ( $0.33 \pm 0.022$  standard error of the mean [SEM]) that was around double that of a typical insect viewing the same regions ( $0.18 \pm 0.025$ ; Figure 3b,c;  $P < 0.001$ ; Student's paired  $t$ -test,  $t = 3.62$ , degrees of freedom [df] = 13). The differences in Euclidean color distance resulted from differences in the long-wavelength channel of each animal. Photon catch models suggest that the Michelson contrast of the hourglass against its black background is greater ( $P < 0.001$ ; Student's paired  $t$ -test,  $t = 10.6$ , df = 13) when viewed by a typical bird long-wavelength photoreceptor (mean = 0.67; SEM = 0.06;  $\lambda_{\max} = 607$  nm; Hart and Vorobyev 2005) than a typical insect long-wavelength photoreceptor (mean = 0.21; SEM = 0.11;  $\lambda_{\max} = 520$  nm; Briscoe and Chittka 2001). Note that Michelson values are not linearly scaled (Cronin 2014).





**Figure 2**

(a) When controlling for bird species, wild birds are ~3 times more likely to attack a black widow model with an hourglass than one without (see Table 1). (b) Birds with longer bills are more likely to attack both an all-black model (solid line, 95% confidence interval in dark gray) and a widow model with an hourglass (dotted line, light gray).

## ANCESTRAL RECONSTRUCTION OF BLACK WIDOW DORSAL COLORATION

### Methods

To infer past selective pressures on black widow coloration, we used the *Latrodectus* phylogeny from Garb 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 4 rate categories (GTR + I + G).

Ancestral state reconstruction was accomplished using the “ace” function in the R package “ape” (Paradis 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.

### 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 4). 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).

## MICROHABITAT CHOICE

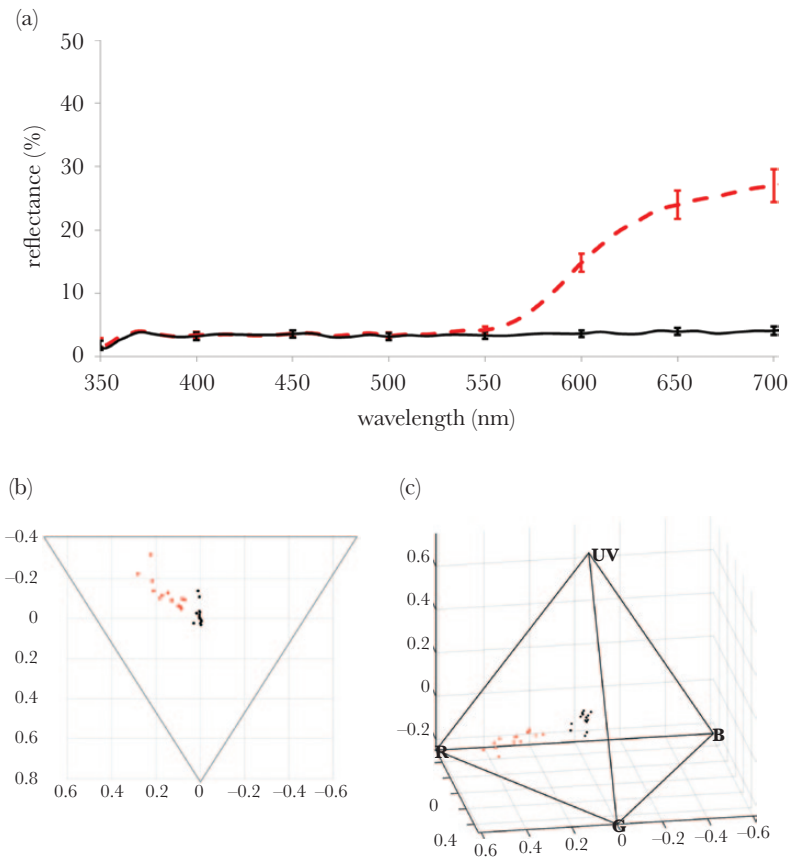
### Methods

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 2 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 (10×10×10 cm; AMAC Plastic Materials Inc., Farmingdale, 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 26×30×50 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 (Supplementary Figure 2). In each widow-tower, 3 false retreats consisting of black spray-painted (The Valspar Corporation, Chicago, IL) 10×10×10 cm boxes (AMAC Plastic Materials Inc.) 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 (Supplementary Figure 2).

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 et al. 2011). At the beginning of an experimental session, one widow was placed at the bottom of a widow-tower. The widow’s 3D position was recorded 72 h after placement, a time that has shown final settlement in other species within the *Latrodectus* genus (Johnson 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.

### 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 5;  $n = 27$ ; chi-square goodness of fit test,  $P < 0.05$ ,  $\chi^2 = 7.53$ ,  $df = 2$ ). 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 3**

(a) Combined spectral reflectance of *Latrodectus mactans* ( $n = 9$ ) and *Latrodectus variolus* ( $n = 5$ ). The error bars show  $\pm$ SE. Dotted gray line (red online) = hourglass, solid black = ventral black of the spider. The hourglass has very little reflectance below  $\sim 550$  nm, and includes no significant UV reflectance. Reflectance data are binned at 10-nm intervals. (b and c) Color constancy distances for a typical insect (b) and a typical bird (c) viewing 14 individual black widows. The black markers represent the color of the black abdomen, whereas the gray (red online) markers represent the color of the red hourglass. Note that that hourglass markers are generally farther away from the black markers in the bird's view than the insect's view. See methods for further information on the photoreceptors used.

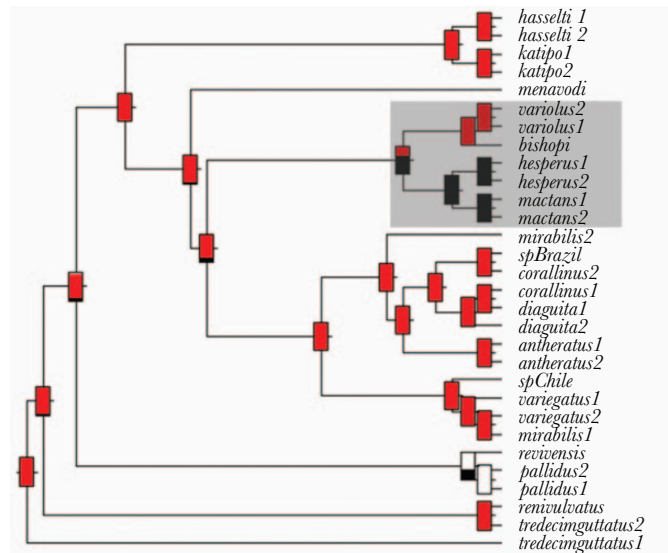
## DISCUSSION

The results from these experiments are consistent with the hypothesis that North American black widow coloration has evolved to be more conspicuous to avian predators than to insect prey. We found that if a wild bird sees a black widow model, they are nearly 3 times more likely to attack an all-black model than one with an hourglass (Figure 2a). Although 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, predation of *Latrodectus* by birds has been observed (Levi 1959; Hódar and Sánchez-Piñero 2002) and may occur if 1) a black widow is not fully hidden by their retreat or 2) if the retreat is disturbed. Additionally black widows are generalist predators, and in some cases, their diet includes many species of insects that are primarily active (and would be subdued) during the day (Hódar and Sánchez-Piñero 2002; Salomon 2011). Although a few animals are capable of seeing color during nocturnal illumination levels (Kelber et al. 2002; Roth and Kelber 2004), color vision may be rare in 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.

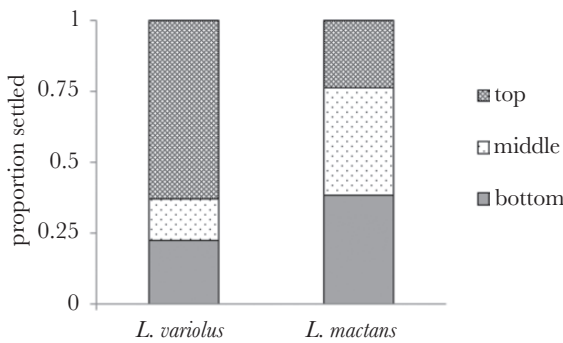
Although we cannot determine if birds are avoiding black widows because of true aposematism, generalized aversion, or neophobia, the positive relationship between beak size and probability of attack suggests that it is adaptive for small birds to avoid attacking black widows. Because the defenses of black widows (web and venom) are only effective at short ranges, black widows are most dangerous to birds with short beaks. We found that birds with the shortest beaks were least likely to attack either model in our study (Figure 2b), and beak size was a better predictor of attack than wing size (Table 1). Together these data suggest that small-beaked birds have been selected to avoid attacking spiders that resemble widows and that deterrence of birds is probably not caused by Batesian mimicry.

The red-and-black coloration used by black widows presents the longest-wavelength photoreceptor of a typical bird ( $\lambda_{\max} = 606$  nm) with a Michelson contrast (0.67) that is greater than that of a typical insect's longest-wavelength photoreceptor ( $\lambda_{\max} = 520$  nm; Michelson contrast = 0.21), which leads to a Euclidean color distance that is 1.83 times larger for birds than insects (Figure 3). Of color combinations that are easily visible to birds, the red-and-black



**Figure 4**

Ancestral reconstruction of dorsal coloration within adult female black widows. The gray 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).



**Figure 5**

Microhabitat choice data for 2 species of black widows that can be found in the same habitats. After 72h, the dorsally colored *Latrodectus variolus* settled in microhabitats that were higher off of the ground than *Latrodectus mactans*.

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 more conspicuous to predators than to prey. An ancestral reconstruction shows that the common ancestor of all black widows likely had dorsal red coloration (Figure 4). At some point during their evolutionary history, 2 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). 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 3D habitats than *L. mactans* (Figure 5), consistent with earlier field observations by Kaston (1970). Because *L. mactans* prefers microhabitats that are relatively close to ground level, *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. Therefore, *L. variolus* has a higher probability that a predator approaches them from below than *L. mactans* and may benefit from a conspicuous dorsal color pattern. 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, neither seems relevant for black widows because no species of North American black widow is considered to be more dangerous than the others, and differences in predators between *L. mactans* and *L. variolus* may be minimal because they are often sympatric (Kaston 1970).

Researchers have often discussed the “cost of conspicuousness” of an aposematic signal as an initial cost imposed by naive predators (Mallet and Singer 1987; Ruxton et al. 2004; Marples et al. 2005). However, conspicuousness carries costs even when an aposematic signal is established. For example, potential prey may use

a conspicuous aposematic signal to avoid the signaler and therefore lower the signaler's foraging rate (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 et al. 1993; Eisner et al. 1998; Eisner T and Eisner M 2000); being conspicuous to these individuals may attract them and lower the fitness of the signaler as a result. 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 nonprimate mammals). 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 toward 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 animals other than the intended receiver influence mimicry and the diversity of aposematic patterns seen in nature.

## SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

## FUNDING

Funding for some of the experimentation was provided by the Duke Biology Department grant-in-aid-of-research.

The authors would like to thank K. Akre, J. Baldwin Fergus, D. Speiser, D. Steinberg, and the Johnsen lab for comments on previous versions of this manuscript. We are grateful for statistical help provided by S. Mukherjee and D. Runcie.

**Handling editor:** Johanna Mappes

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