

Article

Environment-dependent attack rates of cryptic and aposematic butterflies

Brett M. SEYMOUR^{a,b,c,*}, Andrew RAYMUNDO^{b,c}, Kevin J. McGRAW^b,
W. OWEN McMILLAN^c, and Ronald L. RUTOWSKI^b

^aDepartment of Biology, Colorado State University, Fort Collins, CO 80523, USA, ^bSchool of Life Sciences, Arizona State University, Tempe, AZ 85287, USA, and ^cSmithsonian Tropical Research Institute, Panama City, Panama

*Address correspondence to Brett M. Seymore. E-mail: brett.seymore@gmail.com.

Received on 3 July 2017; accepted on 14 September 2017

Abstract

Many organisms have evolved adaptive coloration that reduces their risk of predation. Cryptic coloration reduces the likelihood of detection/recognition by potential predators, while warning or aposematic coloration advertises unprofitability and thereby reduces the likelihood of attack. Although some studies show that aposematic coloration functions better at decreasing attack rate than crypsis, recent work has suggested and demonstrated that crypsis and aposematism are both successful strategies for avoiding predation. Furthermore, the visual environment (e.g., ambient lighting, background) affects the ability for predators to detect prey. We investigated these 2 related hypotheses using 2 well-known visually aposematic species of *Heliconius* butterflies, which occupy different habitats (open-canopy vs. closed-canopy), and one palatable, cryptic, generalist species *Junonia coenia*. We tested if the differently colored butterflies differ in attack rates by placing plasticine models of each of the 3 species in 2 different tropical habitats where the butterflies naturally occur: disturbed, open-canopy habitat and forested, closed-canopy habitat. The cryptic model had fewer attacks than one of the aposematic models. Predation rates differed between the 2 habitats, with the open habitat having much higher predation. However, we did not find an interaction between species and habitat type, which is perplexing due to the different aposematic phenotypes naturally occurring in different habitats. Our findings suggest that during the Panamanian dry season avian predation on perched butterflies is not a leading cause in habitat segregation between the 2 aposematic species and demonstrate that cryptically colored animals at rest may be better than aposematic prey at avoiding avian attacks in certain environments.

Key words: avian attacks, camouflage, *Heliconius*, *Junonia*, light environment, plasticine models, predation, warning coloration.

Many animals face high rates of predation in the wild and have evolved a diverse array of defenses to increase survival (Poulton 1890; Cott 1940; Ruxton et al. 2004; Stevens and Merilaita 2009). One adaptation to avoid predation is camouflage, in which a prey's color pattern blends with that of the visual background (i.e., crypsis), rendering that individual difficult for potential predators to detect (Edmunds 1974; Endler 1984; Cuthill et al. 2005; Stevens and Merilaita 2011; Seymore and Aiello 2015). Another common defensive adaptation is aposematism, in which the characteristics of potential prey animals that are potentially damaging to predators

(e.g., stings, toxins, armor, etc.) are coupled with conspicuous signals to facilitate predator recognition of unprofitable prey (Wallace 1867; Poulton 1890; Ruxton et al. 2004). The functional benefits of both crypsis and aposematism are well documented (Endler 1981; Heiling et al. 2005; Mappes et al. 2005; Speed et al. 2010; Summers et al. 2015); however, comparisons between the 2 visual strategies are lacking (but see Carroll and Sherratt 2013).

Little is known about the differential fitness benefits between these 2 types of defensive coloration, crypsis and aposematism. Does aposematic coloration reduce predation better than crypsis due to

mutual benefits to both the prey (i.e., survival) and predator (i.e., avoiding noxious characteristics; Papageorgis 1975; Guilford 1990; Guilford and Dawkins 1993; Mappes et al. 2005; Saporito et al. 2007)? Until recently there was no direct comparison of attack rates on cryptic and aposematic prey by wild predators in the field. Carroll and Sherratt (2013) used pastry baits with paper model wings and found that aposematic prey and cryptic prey had the same overall attack rates, but that aposematic prey were less fully consumed than cryptic prey. In other words, although the attack rate on aposematic prey and cryptic prey is similar, cryptic prey are more likely to be fully consumed, rather than bitten and released. Hence, there appear to be opportunities for aposematic, but not cryptic, prey to be taste-rejected by predators, leading to higher survival of aposematic prey (Wiklund and Järvi 1982; Pinheiro 1996; Nokelainen et al. 2014).

The intensity of selection from visually hunting predators will not only be a function of unpalatability and predator cognition, but also how coloration and backgrounds are perceived by the visually hunting predators. Perception of prey depends upon several factors including the reflectance of the prey's surface, the behavior of both prey and predator, the ambient lighting, transmission properties of the environment, and predator visual sensitivity (Endler 1990, 1993; Stevens 2013; Hutton et al. 2015). These various determinants of trait perception have led to the hypothesis that the nature of selection on cryptic and warning coloration will be different in disparate environments (Endler 1990, 1992; Stevens and Merilaita 2011). Camouflage depends on the ambient illumination and visual background; therefore, 1 phenotype may be cryptic in 1 set of conditions and very conspicuous in another (Endler and Greenwood 1988; Rojas 2014). Also, Douglas (2013) demonstrated that aposematic butterflies differ in coloration depending on the habitat in which they are found, with tropical understory butterflies exhibiting high achromatic contrast (i.e., black and white), while butterflies that occupy open habitats exhibited highly chromatic contrasts (e.g., yellow and red). However, no study to date has tested attack rates of naturally cryptic individuals and of aposematic species in different habitats. Different habitats should affect predation rates due to visibility of prey (e.g., dense forest vs. open fields), local abundance of predators, environmental effects on conspicuousness (i.e., lighting and visual background), as well as differences in prey abundance and predator experience with specific warning color patterns. Therefore, the environmental context must be considered when assessing the survival advantages of particular "conspicuous" aposematic and "inconspicuous" cryptic phenotypes.

Lepidoptera offer excellent opportunities to comparatively test the environmental factors that affect the adaptive value of crypsis and aposematism (Endler 1984; Nokelainen et al. 2014). Many Lepidoptera, such as the common buckeye butterfly *Junonia coenia*, are profitable prey with inconspicuous coloration when perched (Silberglied et al. 1979; Devries 1987; Pinheiro 1996; Camara 1997), whereas other species such as *Heliconius* butterflies sequester host plant toxins and display a conspicuous warning coloration (Chai 1986; Devries 1987). Both *J. coenia* and *Heliconius* butterflies occur in Panama (Brown 1981; Kozak et al. 2015). Unlike the palatable *J. coenia*, *Heliconius* butterflies contain cyanogenic glycoside toxins (Cardoso and Gilbert 2013), which combined with their conspicuous color patterns leads avian predators to avoid consuming them (Chai 1986; Finkbeiner et al. 2014; Langham 2005). Furthermore, *Heliconius* butterflies exhibit immense color diversity both within and between species and may have up to 5 different aposematic color patterns that are segregated by habitat in 1 forest

(Papageorgis 1975; Devries 1987; Mallet and Gilbert 1995; Thurman and Seymoure 2016). In the lowland rainforest of Panama, 2 aposematic coloration patterns are segregated by habitat, the Postman (yellow, red, and black; comprised of *Heliconius melpomene* and *Heliconius erato*) occurs in open-canopy, disturbed habitats and the Blue-white (blue, white, and black; comprised of *Heliconius cydno* and *Heliconius sapho*) occurs in closed-canopy, undisturbed forest (Estrada and Jiggins 2002). Therefore, these 2 different aposematic groups live in areas with different ambient illumination (brighter and broad spectrum in open-canopy, while darker and rich in green light in closed-canopy), as well as with different avian predators (Endler 1993). Due to the habitat segregation of these aposematic patterns, tests of environmental effects on the effectiveness of aposematic coloration are possible (Endler 1992).

Here, we utilized plasticine models of a cryptic species *J. coenia*, and the 2 species with aposematic color patterns (*H. melpomene* for the Postman mimicry ring and *H. cydno* for the Blue-white mimicry ring) to test 3 sets of hypotheses and predictions where both butterflies and educated predators naturally occur: 1) cryptic and aposematic individuals have evolved coloration to reduce predation and therefore will have similar attack rates; 2) the cryptic species has evolved to be undetected at rest and therefore the cryptic species will have similar attack rates across both habitats; and 3) the aposematic species' warning signals are most effective in their respective habitats and therefore we predict that the Postman will be attacked less in open-canopy while Blue-white will be attacked less in closed-canopy habitats.

Materials and Methods

Model construction

We collected 3 males each of *H. melpomene* (Postman pattern), *H. cydno* (Blue-white pattern), and *J. coenia* in lowland rainforest habitats of central Panama in July 2012 using aerial nets. We then used these males to develop artificial models following the methods of Finkbeiner et al. (2012) and Seymoure and Aiello (2015). The models were constructed using scanned images (Brother MFC-J4510DW Scanner, Brother Industries, Nagoya, Japan) of ventral wing surfaces of each species because individuals of *Heliconius* and *Junonia* perch with their wings closed unless they are thermoregulating or involved in courtship (Brown 1981; Devries 1987). High resolution models were printed onto Whatman filter paper (GE Healthcare Life Sciences, Pittsburgh, PA, USA) with a Brother MFC-J4510DW printer (Brother Industries) and then cut and inserted into the "body," a 2.5-cm long piece of black, non-toxic plastalina modeling plasticine (Craftsmart, Irving, TX, USA), which remains malleable in the field and thereby shows beak marks when attacked by the bill of avian predators (Finkbeiner et al. 2012; Merrill et al. 2012; Seymoure and Aiello 2015).

Model color measurements

To confirm that each model type was visually indistinguishable from the natural butterfly wings, we quantified full-spectrum reflectance and incorporated the data into avian visual threshold models (Vorobyev and Osorio 1998; Maia et al. 2013). We measured the ventral reflectance of the main color patches for each species using 3 male individuals and then measured the same color patches of 3 of each printed model type using a USB2000 Spectroradiometer (Ocean Optics, Dunedin, FL, USA) and Xenon standardized light source (Ocean Optics). Wing color reflectance was measured as the

proportion of a white reference standard (WS-1-SL, Ocean Optics) using a coaxial fiber cable (QR400-7, Ocean Optics). We used avian visual thresholds using the PAVO program within R (Maia et al. 2013; R Core Team 2014) to determine if the artificial wing models accurately represented the coloration of natural wings, as seen through the eyes of birds with both ultraviolet-sensitive (UVS) and violet-sensitive (VS) visual systems (Vorobyev and Osorio 1998; Osorio and Vorobyev 2005). Although the main predators of *Heliconius* are jacamars and tyrant flycatchers (Pinheiro 2011), which have the VS visual system, the predators of *J. coenia* may include predators with either the VS or UVS visual system (Devries 1987). We applied von Kries transformation to account for receptor adaptation and used the default parameters for Weber's fraction (0.05), illumination (D65 irradiance spectrum for standard daylight), background, and cone ratios of $N1=1$, $N2=2$, $N3=2$, $N4=4$ (Hart 2001; Maia et al. 2013). We calculated both achromatic and chromatic just noticeable differences (JNDs) for each main color patch of each model compared with its respective natural butterfly: Postman red, Postman yellow, Postman black, Blue-white white, Blue-white black, Blue-white red, *Junonia* brown, and *Junonia* orange, see Supplementary Figure S1. We did not run JND tests for the blue of the Blue-white mimicry ring because the blue is iridescent and in most cases will be seen as black. It is only at certain angles that a blue hue is reflected from the wing. As we were not able to replicate the iridescence in these paper models, we focused on replicating the black, as this is most likely what predators will see when butterflies are roosting. JNDs represent the ability of a visual system to perceive 2 colors differently, with a JND value of <1 being indistinguishable in ideal conditions (Siddiqi et al. 2004). All comparisons had JNDs of <1 for achromatic and chromatic comparisons for both the V/Vis and UV/Vis visual systems, see Supplementary Figures S2 and S3. Therefore, we inferred that in the eyes of birds the difference in coloration between the models and real butterflies would be minimal if not imperceptible. Furthermore, spectral reflectance curves for each model fit within the natural color variation of each species, see Supplementary Figure S1.

Attack rate experiments

We tested the attack rates of our model types in 2 different habitats in Soberania National Park in Central Panama (9.1° N, 79.7° W). Models were set out in blocks of 3 that included one of each color pattern (i.e., Postman, Blue-white, and *Junonia*). Within each block, models were arranged randomly 1–3 m apart at heights ranging from 0.2 m to 2 m. We tied each model with black string to leaves and branches of rainforest plants. Although we did not specifically control for background, there is no evidence that *Heliconius* individuals or *J. coenia* choose a particular type of vegetation or background for resting (Devries 1987; Mallet and Gilbert 1995). Furthermore, due to the heterogeneity of the vegetation at each site, it is unlikely that a predator would see all 3 models instantaneously. Each block was placed 100 m from the nearest block to reduce the risk of the same bird attacking models as most avian predators of butterflies have home ranges of <1 ha (Buskirk et al. 1972; Karr 1977). Furthermore, it is unlikely that predators learned that the plasticine body was unprofitable due to the few exposures of the plasticine bodies. Learning experiments indicate that birds need more than 3 experiences to learn unpalatability and thus develop avoidance (Skelhorn et al. 2016). As we were testing the efficacy of the coloration of the 3 species of butterflies, we did not manipulate secondary defenses to control for any chemical cue that predators may rely upon. Blocks of models were placed in each habitat type,

open-canopy and closed-canopy, which were categorized by canopy cover (open canopy was defined as having $<70\%$ canopy cover, whereas closed-canopy had $>90\%$ canopy cover; author, unpublished data). Each specific block site was only used once and there were fewer locations in the closed-canopy habitats to place models, so the overall sample size for open-canopy was 99 blocks while closed-canopy was 50 blocks, for a total placement of 447 models. We conducted 8 different 3-day trials from February to April during the dry season in 2013. These experiments took place during the dry season for 2 reasons: because predation rates on insects are higher in the dry than in the wet season and to avoid the potential for rain damage to the models (Kricher 2011). Each model was checked daily (11 AM–4 PM) for 3 days for beak, teeth, and mandible marks (see Finkbeiner et al. 2012; Seymoure and Aiello 2015). Attacked models were removed from the experiment and not replaced, to avoid inflating mortality rates among treatments (Cuthill et al. 2005; Finkbeiner et al. 2012; Merrill et al. 2012). We counted only beak marks (i.e., triangular indentations, see Figure 1) as predatory attacks. Models that disappeared were censored (i.e., included in the attack analyses until removed from the study for non-relevant reasons) in the statistical analysis, because it is impossible to know if the models were removed by an avian predator or a non-relevant force (e.g., curious people, rodents, wind) (Hurlbert 1984). Models that showed evidence of non-avian attacks (i.e., teeth marks and gashes of mammals; small holes of insects) were also censored in the statistical analysis since these attacks were unlikely to have been visually guided and are therefore not a good indicator of color-based predation (Finkbeiner et al. 2012).

Statistical analysis

Differences in attack rates after 72 h were analyzed using Cox proportional-hazards regression (“survival” package) in R (R Core Team 2014). Missing models and non-avian attacks were censored in the Cox proportional-hazards regression. Model type (i.e., Postman, Blue-white, and cryptic), habitat (i.e., open and closed), date of trial, and block were included in the Cox analysis. Both date of trial and block were analyzed as random factors. We also calculated the effect sizes with odds ratios (OR), where a value of 1.00 indicates that 2 models have identical probabilities of being attacked. We must note that the OR test assumes that all model types have an equal chance of being attacked and because we removed models once they were attacked, we violated a key test assumption. However, we analyzed both the OR test and the Cox proportional hazards 2 ways: 1) with all attacks included in the



Figure 1. Examples of artificial models placed in the fields with marks interpreted as beak marks from attacks by avian predators on plasticine-paper models. Arrows point to beak marks. Left, a beak mark on the plasticine abdomen of a Postman model; central, a beak mark on the plasticine abdomen of a Blue-white model; right, the wing pulled from the body of the *J. coenia* model.

Table 1. Number of models that displayed evidence of avian and non-avian attacks, or went missing during the trials for each species and habitat

Species	Open				Closed			
	N	Avian attack	Non-avian attack	Missing	N	Avian attack	Non-avian attack	Missing
<i>H. melpomene</i>	99	20	3	10	50	3	3	1
<i>H. cydno</i>	99	15	0	8	50	5	1	1
<i>J. coenia</i>	99	9	2	14	50	2	1	0

The number of models placed is represented by N.

analysis regardless of whether the model was attacked first in the block or after an initial attack on another model in the same block; and 2) with only the models in a block that were attacked first and other sequential attacks were censored. Both statistical approaches resulted in the same test statistics and thus we conclude that although the methods may have violated a statistical assumption of the OR test, our findings are rigorous.

Results

Over the 8 different trials, all of which lasted 3 days, 12.1% (54/447) of the models showed evidence of attack by birds. Avian attack rates in the open habitat were 14.8% (44/297) and in the closed habitat were 6.7% (10/150). Attacks by non-avian predators (e.g., rodents and insects) contributed another 2.2% (10/447), while 7.6% (34/447) of the models were missing (Table 1). Lastly, the open habitat had 10.8% (32/297) of the models missing while the closed only had 1.3% (2/150). The high rates of missing models in the open habitat are due to areas of forest being clear cut and removing 15 models, 5 of each model type. We included these missing models into our analysis because the clear cutting occurred after day 1, thus allowing for the use of attack data from these models for at least 1 day.

Model survivorship curves differed significantly by model type (Cox regression, $F = 2.049$, $P = 0.040$; Figure 2A) and habitat (Cox regression, $F = 2.536$, $P = 0.011$; Figure 2B), but not with placement date (Cox regression, $F = 1.784$, $P = 0.074$), nor the random factor of block (Cox regression, $\chi^2 = 0.07$, $P = 0.53$). Also, the model statistic was the same regardless if only the first model attacked was included in the model when compared with having all attacks in each block included. Furthermore, there was not an interaction between model type and habitat (Cox regression, $F = 0.533$, $P = 0.594$). Pairwise comparisons revealed that independent of habitat, aposematically colored *H. melpomene* models were attacked more often than cryptically colored *J. coenia* models (Wald = 10.18, $df = 2$, $P = 0.006$, OR = 2.290), but aposematically colored *H. melpomene* had similar attack rates to aposematically colored *H. cydno* models (Wald = 5.26, $df = 2$, $P = 0.061$, OR = 1.177). *Heliconius cydno* and *J. coenia* models also had similar attack rates (Wald = 4.73, $df = 2$, $P = 0.094$, OR = 1.945). Also, the number of attacks on *H. melpomene* differed between habitat types with much higher predation in the respective, open habitat of *H. melpomene* (Wald = 4.48, $df = 1$, $P = 0.034$, OR = 3.966; Supplementary Figure S4), while the number of attacks on the other 2 species did not differ between habitats (*H. cydno*: Wald = 0.840, $df = 1$, $P = 0.358$, OR = 1.607; *J. coenia*: Wald = 1.38, $df = 1$, $P = 0.240$, OR = 2.4; Supplementary Figure S4).

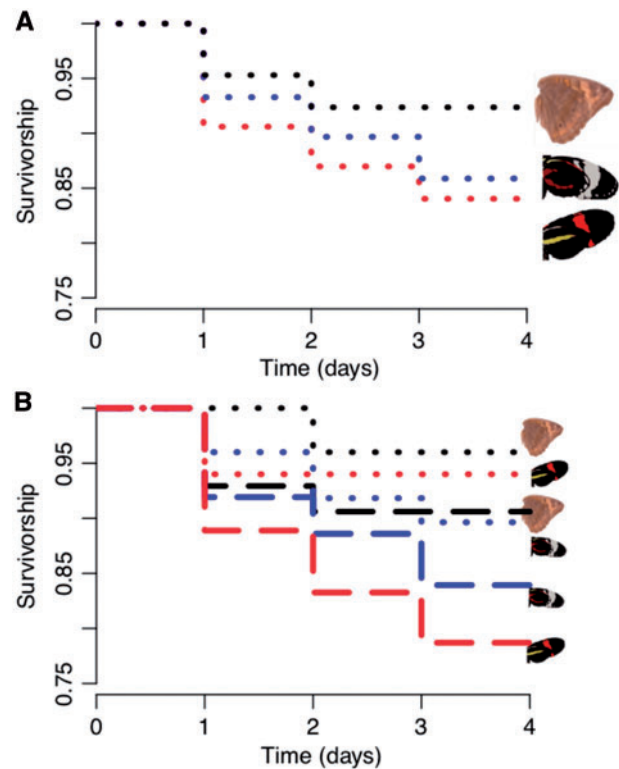


Figure 2. Survival curves for the 3 different models. Red represents postman *H. melpomene*, blue represents Blue-white *H. cydno*, and brown represents the cryptic model *J. coenia*. (A) Combined habitat survival curves for each morph. (B) Individual survival curves for each morph in each habitat. Long dashes represent survival in the open habitat while dots represent survival in the closed habitat.

Discussion

Previous research has shown that both cryptic individuals and aposematic individuals have similar attack rates in artificial prey (Carroll and Sherratt 2013). Here, we demonstrate that attack rates on 2 different aposematic species (*Heliconius*) and cryptic (*Junonia*) individuals depend on coloration as well as the environment. We found that the aposematic Postman models were attacked more than the cryptic model, yet the 2 aposematic color patterns had similar attack rates. Furthermore, the attack rates differed among habitats with more attacks occurring in the open habitat than in closed habitat. Our results, along with Carroll and Sherratt's (2013) results, indicate that aposematic theory needs to include factors other than just conspicuousness and unpalatability.

Heliconius butterflies are aposematic and several studies have demonstrated that avian predators recognize the visual warning signals of *Heliconius* to avoid attacking individuals (Chai 1986, 1996;

Chai and Srygley 1990; Langham 2004, 2005). Previous research on the avian community in central Panama has revealed that the closed habitat has different insectivorous bird species compared with open and edge habitats (Karr 1977; Samuel et al. 1985; Poulin and Lefebvre 1996; Robinson et al. 2000). However, at the family level, the composition is similar with the main Lepidoptera predators being non-migratory flycatchers, jacamars, and warblers (see Poulin and Lefebvre 1996; Robinson et al. 2000). The likely avian predators of *Heliconius* and other tropical butterflies are flycatchers and jacamars (Pinheiro 1996), which often aerially attack prey at the thorax and then either consume palatable prey or taste reject chemically defended prey (Pinheiro 2011). Thus, our study is complicated at 2 levels: 1) our models were sedentary and may not be the best surrogate for naturally occurring attack rates and 2) our models did not differ in palatability and we could not assay taste rejection by avian predators. Taste rejection is likely an adaptation to find palatable mimics of aposematic prey and the act of taste rejection has been shown to leave butterflies intact and capable of flight (Wiklund and Järvi 1982; Sillen-Tullberg 1985; Pinheiro 1996, 2011). Therefore, although we found that the cryptic species had fewer attacks than the aposematic Postman species, we were not able to determine whether the aposematic species would have been taste rejected since the bodies were plasticine. It is likely that the survival rates of all 3 species are similar in wild butterflies due to taste rejection by birds. In fact, Carroll and Sherratt (2013) demonstrated that artificial models made to be unpalatable with quinine pastry baits, were attacked at the same rate as palatable, cryptic pastry bait models, but that the unpalatable pastry baits were taste rejected more often. Future studies to test taste rejection in these species of butterflies in the wild are needed to better understand the role of predator behavior in selecting for aposematic and cryptic phenotypes.

Our study replicated components of the study by Merrill et al. (2012) in that we used plasticine models of Postman and Blue-white butterflies in Panama to determine if predation rates differed between aposematic morphs in different habitats. Although we found similar results as Merrill et al. (2012) for the overall study in that butterflies were not less likely to be attacked in their respective environment, we found that overall attack rate did differ between forest edge and forest habitats, whereas Merrill et al. (2012) did not find differences in attack rates dependent upon habitat. Our findings may differ from Merrill et al. (2012) because we tested predation during the dry season instead of the wet season. Avian predation has been reported to increase during the dry season due to lower availability of prey, which may mean that aposematic prey are attacked more during the dry season than in the wet season (Kricher 2011). In fact, we observed an attack rate that was 3 times that recorded by Merrill et al. (2012; 12% compared with 4%), even though the overall methods were very similar. Seasonal differences in attack rates have also been reported by Mappes et al. (2014), who found that the attack rates of cryptic and aposematic larvae in Finland varied with season. Specifically, Mappes et al. (2014) attributed the seasonal attack differences between cryptic and aposematic larvae to seasonal differences in the prior experiences of avian predators. Naïve fledglings attacked more aposematic prey than cryptic prey, but later in the year when all birds were experienced, the cryptic prey were attacked more than aposematic prey. In our study, it is possible that differences in predation rates between aposematic and cryptic morphologies were due to bird age and experience. Both tyrant flycatchers and jacamars have breeding seasons that begin at the transition from wet season to dry season and thus naïve

fledglings begin foraging during the dry season and may have not learned to avoid aposematic species (Skutch 1968; Hoyo et al. 2004).

There were more overall attacks for each species in the open habitat, although there was only a significant difference for *H. melpomene*. This finding is most likely due to visibility and predator composition. The closed, forested site where models were placed was thick with vegetation and therefore it may have been harder for birds to detect even the conspicuous models. Also, predator composition varies between the 2 habitats and the forest edge habitat has high abundance of insectivorous birds such as tyrant flycatchers (del Hoyo et al. 2004).

The Postman coloration was attacked more in its respective habitat than in the habitat where it does not reside. This is contrary to our predictions as we predicted that predation on aposematic models would be lower where the aposematic model is common due to experienced predators as has been supported by previous research (Mallet and Barton 1989; Merrill et al. 2012). As stated previously, this suggests that avian predators are likely attacking aposematic individuals and then deciding whether to consume or reject the prey dependent upon chemical defenses (Wiklund and Järvi 1982; Sillen-Tullberg 1985; Pinheiro 1996; Pinheiro 2011; Carroll and Sherratt 2013). *Heliconius* species have many palatable mimics that may be rewarding avian predators that test the palatability of prey items (Pinheiro 1996, 2007, 2011). And if the palatable mimics are segregated by habitat like their aposematic model (i.e., Postman butterflies), then predators may be searching for individuals with the Postman coloration. Furthermore, the Postman has high chromatic contrast (red, yellow, and black color pattern) and thus is highly noticeable in well-lit environments like edge habitats and may be easier to detect by avian predators in the edge habitat (Douglas 2013). Further research into the rates of taste rejection in aposematic species is needed to understand the evolutionary processes behind warning coloration and mimicry.

Plasticine models have been used to test many hypotheses explaining differences in morphology, as well as hypotheses relative to the ecology and evolution of predator-prey interactions (Papageorgis 1975; Cuthill et al. 2005; Finkbeiner et al. 2012; Seymoure and Aiello 2015). However, in several such studies, the plasticine model manipulations done to address the questions they proposed are artificial and do not resemble any natural prey item (see Cuthill et al. 2005; Carroll and Sherratt 2013) or are drastically different from the natural coloration (see Finkbeiner et al. 2014; Seymoure and Aiello 2015). It is conceivable that this may lead to attack rates that are higher than would occur with natural coloration. Hence, the comparatively low predator attack rates that we observed might be due to the relatively natural appearance of the plasticine models that we used.

Our findings here suggest that both aposematism and cryptic coloration have low attack rates in the wild. However, the plasticine models are a surrogate for wild butterflies and may not be equally representative of the attack rates for living cryptic and aposematic individuals. Most prey items move, especially butterflies, and the models used in this study were static, so perhaps predation rates between cryptic and aposematic animals differ when movement is included. In fact, cryptic organisms are hypothesized to move less than conspicuous organisms because predators can use movement to detect prey (Stevens and Merilaita 2011).

In conclusion, our study suggests that both aposematic coloration and cryptic coloration can be adaptive strategies for avoiding predation at rest as all models had low attack rates. The findings

suggest that the form of aposematic coloration and the habitat (i.e., open-canopy vs. closed-canopy) in which an organism resides affects the predation rate. All 3 color forms were attacked more in the open habitat, which is most likely due to visibility and perhaps greater abundance of predators. Furthermore, the more chromatic aposematic species was attacked more than the cryptic species. Lastly, this study highlights the need for further research into the tradeoffs of crypsis and aposematism including using avian visual models to determine how different habitats (open vs. closed) affect the conspicuousness of color patterns. Why do some animals evolve crypsis while others evolve aposematism, if both have similar rates in survival? Future work studying the role of life history (e.g., dispersal, mobility, and host plants) and predation risk in the context of crypsis and aposematism is needed to understand the selection pressures leading to crypsis or aposematism.

This research was a product of the Arizona State University and Smithsonian Tropical Research Institute Partnership. We are grateful for resources and logistical support from both Arizona State University and the Smithsonian Tropical Research Institute. Furthermore, we are indebted to Lindsey Seymoure for her help with developing the butterfly models and to Beryl Jones for help in the field. The McMillan Laboratory at the Smithsonian Tropical Research Institute and the McGraw and Rutowski Laboratories were essential to the success of this study by providing feedback through the entire process. Finally, this manuscript was greatly strengthened by anonymous reviewers and we thank them for their time and constructive criticisms.

Funding

This research was a product of the Arizona State University and Smithsonian Tropical Research Institute Partnership and was funded internally.

Supplementary Material

Supplementary material can be found at <https://academic.oup.com/cz>.

References

- Brown KSJ, 1981. The biology of *Heliconius* and related genera. *Annu Rev Entomol* 26:427–456.
- Buskirk WH, Powell GVN, Wittenberger JF, Ruth E, 1972. Interspecific bird flocks in tropical highland Panama. *Auk* 89:612–624.
- Camara MD, 1997. Predator responses to sequestered plant toxins in buckeye caterpillars: are tritrophic interactions locally variable? *J Chem Ecol* 23: 2093–2106.
- Cardoso MZ, Gilbert LE, 2013. Pollen feeding, resource allocation and the evolution of chemical defence in passion vine butterflies. *J Evol Biol* 26: 1254–1260.
- Carroll J, Sherratt TN, 2013. A direct comparison of the effectiveness of two anti-predator strategies under field conditions. *J Zool* 291:279–285.
- Chai P, 1986. Field observations and feeding experiments on the responses of rufous-tailed jacamars *Galbula ruficauda* to free-flying butterflies in a tropical rainforest. *Biol J Linn Soc* 29:161–189.
- Chai P, 1996. Butterfly visual characteristics and ontogeny of responses to butterflies by a specialized tropical bird. *Biol J Linn Soc* 59:37–67.
- Chai P, Srygley RB, 1990. Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. *Am Nat* 135:748–765.
- Cott HB, 1940. *Adaptive Coloration in Animals*. London: Methuen.
- Cuthill IC, Stevens M, Sheppard J, Maddocks T, Párraga CA et al., 2005. Disruptive coloration and background pattern matching. *Nature* 434: 72–74.
- Devries PJ, 1987. *The Butterflies of Costa Rica and Their Natural History*. Vol. I: Papilionidae, Pieridae, Nymphalidae. Princeton: Princeton University Press.
- Douglas JM, 2013. *Ambient Light Environment and the Evolution of Brightness, Chroma, and Perceived Chromaticity in the Warning Signals of Butterflies*. Tempe: Arizona State University.
- Edmunds M, 1974. *Defence in Animals*. Englewood Cliffs (NJ): Prentice Hall Press.
- Endler JA, 1981. An overview of the relationships between mimicry and crypsis. *Biol J Linn Soc* 16:25–31.
- Endler JA, 1984. Progressive background in moths, and a quantitative measure of crypsis. *Biol J Linn Soc* 22:187–231.
- Endler JA, 1992. Signal conditions and the direction of evolution. *Am Nat* 139:S125–S153.
- Endler JA, 1993. The color of light in forests and its implications. *Ecol Monogr* 63:1–27.
- Endler JA, 1990. On the measurement and classification of colour in studies of animal colour patterns. *Biol J Linn Soc* 41:315–352.
- Endler JA, Greenwood JJD, 1988. Frequency-dependent predation, crypsis and aposematic coloration. *Philos Trans R Soc Lond Ser B* 319:505–523.
- Estrada C, Jiggins CD, 2002. Patterns of pollen feeding and habitat preference among *Heliconius* species. *Ecol Entomol* 27:448–456.
- Finkbeiner SD, Briscoe AD, Reed RD, 2012. The benefit of being a social butterfly: communal roosting deters predation. *Proc Biol Sci* 279:2769–2776.
- Finkbeiner SD, Briscoe AD, Reed RD, 2014. Warning signals are seductive: relative contributions of color and pattern to predator avoidance and mate attraction in *Heliconius* butterflies. *Evolution* 68:3410–3420.
- Guilford T, 1990. The Evolution of aposematism. In: Evans DL, Schmidt JO, editors. *Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*. Albany: State University of New York Press, 23–62.
- Guilford T, Dawkins MS, 1993. Are warning colors handicaps? *Evolution* 47: 400–416.
- Heiling AM, Chittka L, Cheng K, Herberstein ME, 2005. Colouration in crab spiders: substrate choice and prey attraction. *J Exp Biol* 208:1785–1792.
- Hoyo JD, Elliot A, Christie D, 2004. *Handbook of the Birds of the World*. Barcelona: Lynx Edicions.
- Hurlbert S, 1984. Pseudoreplication and the designs of ecological field experimentation. *Ecol Mono* 54:187–211.
- Hutton P, Ligon RA, McGraw KJ, Seymoure BM, Simpson RK, 2015. Dynamic color communication. *Curr Opin Behav Sci* 6:41–49.
- Karr JR, 1977. Ecological correlates of rarity in a tropical forest bird community. *Auk* 94:240–247.
- Kozak KM, Wahlberg N, Neild A, Dasmahapatra KK, Mallet J et al., 2015. Multilocus species trees show the recent adaptive radiation of the mimetic *Heliconius* butterflies. *Syst Biol* 0:1–20.
- Kricher J, 2011. *Tropical Ecology*. Princeton (NJ): Princeton University Press.
- Langham GM, 2004. Specialized avian predators repeatedly attack novel color morphs of *Heliconius* butterflies. *Evolution* 58:2783–2787.
- Langham GM, 2005. Rufous-tailed jacamars and aposematic butterflies: do older birds attack novel prey? *Behav Ecol* 17:285–290.
- Maia R, Eliason CM, Bitton PP, Doucet SM, Shawkey MD, 2013. pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol Evol* 4:906–913.
- Mallet J, Barton NH, 1989. Strong natural selection in a warning-color hybrid zone. *Evolution* 43:421–431.
- Mallet J, Gilbert LE, 1995. Why are there so many mimicry rings? Correlations between habitat, behaviour and mimicry in *Heliconius* butterflies. *Biol J Linn Soc* 55:159–180.
- Mappes J, Kokko H, Ojala K, Lindström L, 2014. Seasonal changes in predator community switch the direction of selection for prey defences. *Nat Commun* 5:5016.
- Mappes J, Marples N, Endler JA, 2005. The complex business of survival by aposematism. *Trends Ecol Evol* 20:598–603.
- Merrill RM, Wallbank RWR, Bull V, Salazar PC, Mallet J et al., 2012. Disruptive ecological selection on a mating cue. *Proc Biol Sci* 279: 4907–4913.

- Nokelainen O, Valkonen J, Lindstedt C, Mappes J, 2014. Changes in predator community structure shifts the efficacy of two warning signals in Arctiid moths. *J Anim Ecol* 83:598–605.
- Osorio D, Vorobyev M, 2005. Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. *Proc Biol Sci* 272: 1745–1752.
- Papageorgis C, 1975. Mimicry in neotropical butterflies: why are there so many different wing-coloration complexes in one place? *Am Sci* 63: 522–532.
- Pinheiro CEG, 1996. Palatability and escaping ability in neotropical butterflies: tests with wild kingbirds (*Tyrannus melancholicus*, Tyrannidae). *Biol J Linn Soc* 59:351–365.
- Pinheiro CEG, 2007. Asynchrony in daily activity patterns of butterfly models and mimics. *J Trop Ecol* 23:119.
- Pinheiro CEG, 2011. On the evolution of warning coloration, Batesian and Müllerian mimicry in Neotropical butterflies: the role of jacamars (Galbulidae) and tyrant-flycatchers (Tyrannidae). *J Avian Biol* 42:277–281.
- Poulin B, Lefebvre G, 1996. Dietary relationships of migrant and resident birds from a humid forest in central Panama. *Auk* 113:277–287.
- Poulton EB, 1890. *The Colour of Animals: Their Meaning and Use, Especially Considered in the Case of Insects*. New York (NY): D. Appleton and Company.
- R Core Team, 2014. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Robinson WD, Brawn JD, Robinson SK, 2000. Forest bird community structure in central Panama: influence of spatial scale and biogeography. *Ecol Monogr* 70:209–235.
- Rojas B, 2014. Differential detectability under varying light environments: an alternative explanation for the maintenance of polymorphic warning signals? *Behav Process* 109:164–172.
- Ruxton GD, Sherratt TN, Speed MP, 2004. *Avoiding Attack: the Evolutionary Ecology of Crypsis, Warning Signals & Mimicry*. UK: Oxford University Press.
- Samuel MD, Pierce DJ, Garton EO, 1985. Identifying areas of concentrated use within the home range. *J Anim Ecol* 54:711–719.
- Saporito RA, Zuercher R, Roberts M, Gerow KG, Donnelly MA, 2007. Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia* 2007:1006–1011.
- Seymoure BM, Aiello A, 2015. Keeping the band together: evidence for false boundary disruptive coloration in a butterfly. *J Evol Biol* 28:1618–1624.
- Siddiqi A, Cronin T, Loew E, Vorobyev M, Summers K, 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. *J Exp Biol* 207:2471–2485.
- Silberglied RE, Aiello A, Lamas G, 1979. Neotropical butterflies of the genus *Anartia*: systematics, life histories and general biology (Lepidoptera: Nymphalidae). *Psyche A J Entomol* 86:219–260.
- Sillen-Tullberg B, 1985. Higher survival of an aposematic than of a cryptic form of a distasteful bug. *Oecologia* 67:411–415.
- Skellhorn J, Halpin CG, Rowe C, 2016. Learning about aposematic prey. *Behav Ecol* 27:955–964.
- Skutch AF, 1968. The nesting of some Venezuelan birds. *Condor* 70:66–82.
- Speed MP, Brockhurst MA, Ruxton GD, 2010. The dual benefits of aposematism: predator avoidance and enhanced resource collection. *Evolution* 64: 1622–1633.
- Stevens M, 2013. *Sensory Ecology*. Oxford: Oxford University Press.
- Stevens M, Merilaita S, 2009. Animal camouflage: current issues and new perspectives. *Philos Trans R Soc Lond B Biol Sci* 364:423–427.
- Stevens M, Merilaita S, 2011. *Animal Camouflage: Mechanisms and Function*. Cambridge: Cambridge University Press.
- Summers K, Speed MP, Blount JD, Stuckert A, 2015. Are aposematic signals honest? A review. *J Evol Biol* 28:1583–1599.
- Thurman T, Seymoure BM, 2016. A bird's eye view of two mimetic tropical butterflies: coloration matches predator's sensitivity. *J Zool* 298: 159–168.
- Vorobyev M, Osorio D, 1998. Receptor noise as a determinant of colour thresholds. *Proc Biol Sci* 265:351–358.
- Wallace AR, 1867. Mimicry and other protective resemblances among animals. *Westminster Foreign Q Rev* 32:1–43.
- Wiklund C, Järvi T, 1982. Survival of distasteful insects after being attacked by naive birds: a reappraisal of the theory of aposematic coloration evolving through individual selection. *Evolution* 36:998–1002.