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# Complex dynamics underlie the evolution of imperfect wing pattern convergence in butterflies

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Adaptive radiation is characterized by rapid diversification that is strongly associated with ecological specialization. However, understanding the evolutionary mechanisms fueling adaptive diversification requires a detailed knowledge of how natural selection acts at multiple life-history stages. Butterflies within the genus *Adelpha* represent one of the largest and most diverse butterfly lineages in the Neotropics. Although *Adelpha* species feed on an extraordinary diversity of larval hosts, convergent evolution is widespread in this group, suggesting that selection for mimicry may contribute to adaptive divergence among species. To investigate this hypothesis, we conducted predation studies in Costa Rica using artificial butterfly facsimiles. Specifically, we predicted that nontoxic, palatable *Adelpha* species that do not feed on host plants in the family Rubiaceae would benefit from sharing a locally convergent wing pattern with the presumably toxic Rubiaceae-feeding species via reduced predation. Contrary to expectations, we found that the presumed mimic was attacked significantly more than its locally convergent model at a frequency paralleling attack rates on both novel and palatable prey. Although these data reveal the first evidence for protection from avian predators by the supposed toxic, Rubiaceae-feeding *Adelpha* species, we conclude that imprecise mimetic patterns have high costs for Batesian mimics in the tropics.

**KEY WORDS:** *Adelpha*, imperfect Batesian mimicry, palatability, predation.

Convergent evolution is widespread in animal lineages where unrelated species independently evolve similar traits that improve their fitness. This process usually occurs in independent populations inhabiting like environments, which implies natural selection as its underlying driver (Conte et al. 2012). Phenotypic convergence, or the process by which individuals have evolved to share the same appearance, is especially common in lepidopterans (butterflies and moths) whose wing patterns display remarkably similar markings despite convergent individuals sharing an ancestral form with dissimilar features.

Often, unrelated butterfly species in the same geographic area will converge on the same wing pattern to collectively advertise warning signals toward predators (Brower 1958; Benson

1972; Papageorgis 1975; Mallet and Gilbert 1995), which is especially effective when both butterflies are toxic or carry a capture cost. This adaptation—commonly defined as mimicry—provides enhanced protection from predators and is beneficial to numerous groups of butterflies in both tropical and temperate regions. Butterflies can be mimetic in a Müllerian or Batesian context, where Müllerian individuals have traits (e.g., chemical defenses) that impose a cost on would-be predators, while Batesian mimics benefit from sharing the same warning phenotype but do not have the traits (e.g., toxins) that impose a cost on predators (Bates 1862; Müller 1879; Ruxton et al. 2004).

Mimetic traits can arise during or soon after events of rapid diversification, when natural selection drives divergence in response to ecological opportunity (Schluter 2000; Gavrillets and Losos 2009; Losos 2010; Ebel et al. 2015). Because many clades of phytophagous insects (including butterflies) share a close

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relationship with their host plants, shifts in host plant use may cause increases in diversification rates (Janz et al. 2006; Fordyce 2010) as well as changes in caterpillar and adult defenses. Over time, herbivores that feed on host plants employing defensive secondary toxic compounds evolve resistance, and might subsequently evolve aposematic (warning) patterns on their wings that are quickly learned by predators (Ruxton et al. 2004). As a result, such effective warning patterns may be mimicked by both related and unrelated species, giving rise to further speciation and diversification (Willmott and Mallet 2004; Jiggins 2008; Mallet 2009). In addition, mimetic traits themselves can cause rapid diversification, and convergence in different geographic areas onto different shared warning color patterns can lead to divergence between species, races, or even morphs of the same species between geographic areas.

Butterflies from the genus *Adelpha* are among the largest and most diverse butterfly lineages in the neotropics, with increased species richness at lower latitudes near equatorial regions (Willmott 2003). Convergent evolution in *Adelpha* is widespread (Aiello 1984; Willmott 2003; Mullen et al. 2011; Ebel et al. 2015) and many sympatric species share nearly identical wing patterns, suggesting this genus comprises multiple mimicry complexes (see Fig. S1 for examples of convergence). Moreover, *Adelpha* species feed on an extraordinary diversity of host plants, and it has been hypothesized that mimicry occurs between palatable generalists and (presumably) unpalatable specialists that feed exclusively on toxic plants from the Rubiaceae family (Aiello 1984; Fig. 2 in Ebel et al. 2015). Many Rubiaceae plants produce alkaloid compounds known to repel herbivores (Schmeller and Wink 1998; Soto-Sobenis et al. 2001; Kessler and Baldwin 2002), therefore species whose larvae feed on Rubiaceae have been hypothesized to serve as toxic, unpalatable models for mimetic species.

Here, we test mimicry theory in sympatric *Adelpha* butterflies by investigating whether non-Rubiaceae-feeding *Adelpha* benefit from sharing a locally convergent wing pattern with a Rubiaceae-feeding species, using artificial butterflies in predation studies in Costa Rica. We focus on two species with convergent wing patterns: *Adelpha iphichlus*, a Rubiaceae-feeding specialist and the presumed toxic model, and *Adelpha serpa*, a non-Rubiaceae-feeding generalist hypothesized to be the non-toxic Batesian mimic. Both species have remarkably similar wing patterns (Fig. 1) and are found geographically in the same location. We predict that *A. iphichlus* and *A. serpa* will experience reduced predation rates compared to novel and known palatable prey under the assumption that mimicry is effective within this group. We also test base predation rates on various local palatable prey species to gain insight on predator behavior toward palatable prey that vary in abundance and detectability. Our results not only provide the first evidence for unpalatability among Neotrop-

ical *Adelpha* but also suggest, in contrast to mimicry theory, that predators are able to finely discriminate among locally convergent wing patterns.

## Methods

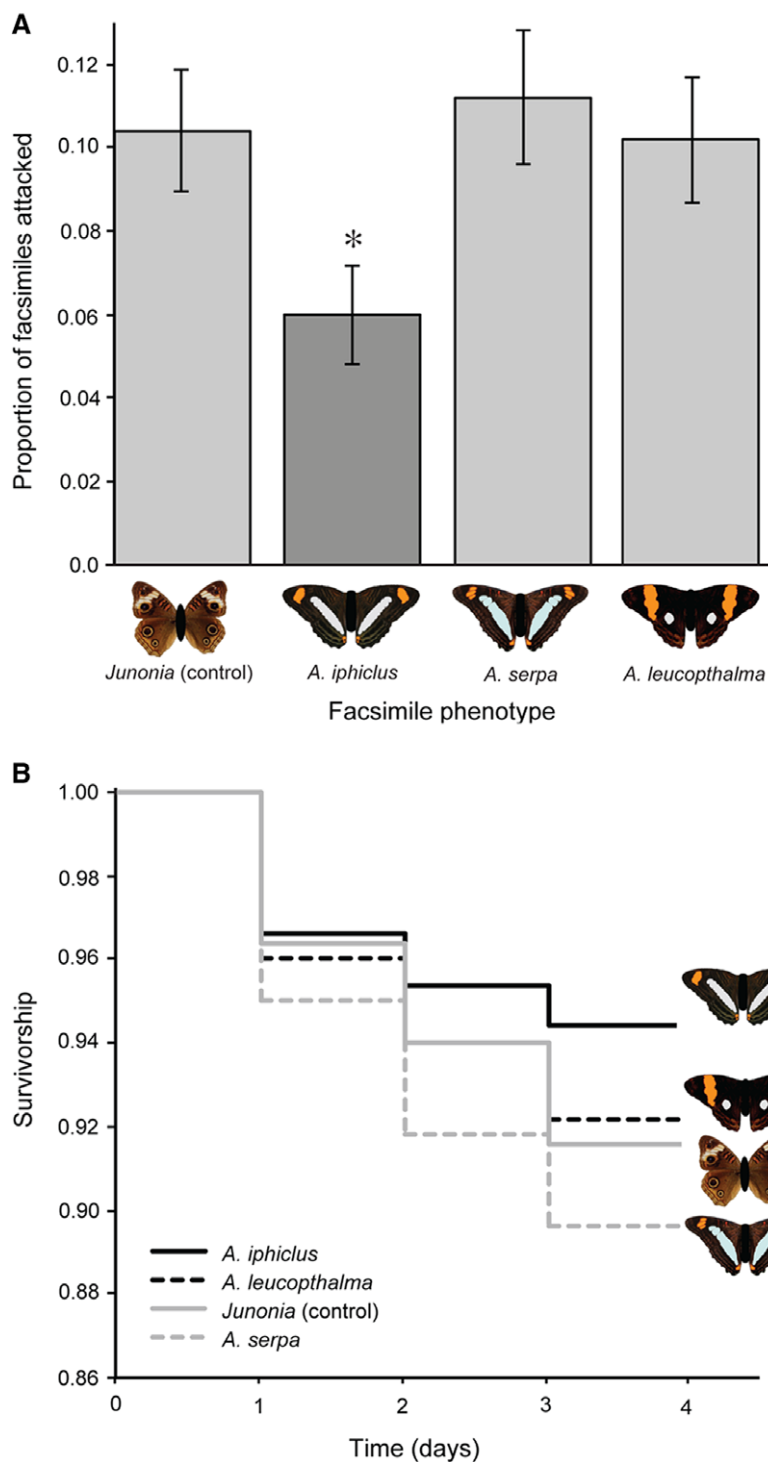
### FIELD SITE

All field experiments took place at the Organization for Tropical Studies' La Selva Biological Reserve in Sarapiquí, Costa Rica (10°25'28" N, -84°0'18" W). This reserve is primarily composed of old-growth and secondary-growth Caribbean tropical lowland forest, with patches of abandoned plantations and open pasture. This field site was chosen based on the abundance and accessibility of *Adelpha* butterflies and avian predators. An initial predation study to determine a suitable control species was carried out during November 2015 in the rainy season, and the primary mimicry study was carried out during April–May 2016 at the end of the dry season. Similar studies have experienced success with butterfly predation experiments at this field site during this time of year (Finkbeiner et al., 2012, 2014).

### ARTIFICIAL BUTTERFLY FACSIMILE PRODUCTION

We used artificial butterfly models (facsimiles) to measure predator response to specific butterfly phenotypes. Butterfly facsimiles were designed following the protocol in Finkbeiner et al. (2012), where natural butterfly wings were referenced to create visually accurate paper wings. Reflectance measurements were taken with an Ocean Optics USB2000 fiber optic spectrometer, bifurcating fiber cable (R400-7-UV-vis Ocean Optics, Winter Park, FL), and a deuterium-halogen tungsten lamp as a standardized light source (Model MINIDT1000-027; Analytical Instrument Systems, Flemington, NJ). A white spectralon standard (WS-1-SL; Labsphere, North Sutton, NH) was used during calibration approximately every five measurements, and the detecting fiber was placed in a probe holder at a 45° angle to the plane of the butterfly wing. The facsimiles were designed to resemble butterflies with wings open at rest (i.e., basking), showing the dorsal side only. In general, *Adelpha* species rest with their wings folded on a downward angle so that the wings take the shape of triangles; therefore, the facsimiles imitated this particular posture (Figs. 1 and S2).

To determine the similarity between artificial and natural wing spectra, the quantum catches for stimuli (Kelber et al. 2003), and then the discriminabilities between species and between artificial butterfly wings and natural wing reflectance spectra were calculated using tetrachromatic bird-vision models from Vorobyev and Osorio (1998). The comparisons were made using the blue tit (*Cyanistes caeruleus*) and chicken (*Gallus gallus*) cone sensitivities, which represent the ultraviolet- and violet-type avian visual systems, respectively. For chicken, we used the behaviorally



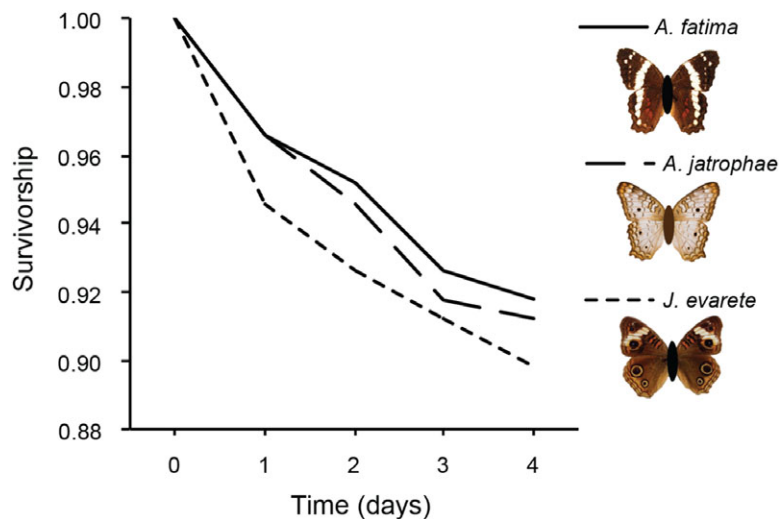
**Figure 1.** Artificial butterfly facsimiles experience bird predation in field studies. (A) Shown are the proportions of facsimiles attacked (total  $n = 2000$ : 500 of each phenotype). There is no difference in predation between the palatable control (*Junonia evarete*), phenotypically convergent *Adelpha serpa*, and allopatric (novel) *Adelpha leucothalma* (all  $P > 0.6$ ). Rubiaceae-feeding *Adelpha iphicus* was attacked significantly less compared to the other three phenotypes, where  $*P < 0.03$  for all comparisons (using a general linear model with Poisson distribution). Error bars include 95% CIs. (B) Survivorship curves show similar results where *A. iphicus* has the highest overall survivorship compared to the other butterfly phenotypes ( $*P < 0.02$ ; Cox proportional-hazards regression model). *Adelpha serpa*, *A. leucothalma*, and *J. evarete* survivorship did not differ significantly from one another (all  $P > 0.7$ , marked with NS).

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**Table 1.** Results from discriminability calculations using high light intensity and daylight or forest shade irradiance.

JND Comparisons		<i>A. iphiclus</i> White versus Facsimile White	<i>A. serpa</i> White versus Facsimile White	<i>A. iphiclus</i> White versus <i>A. serpa</i> White	<i>A. iphiclus</i> Facsimile White versus <i>A. serpa</i> Facsimile White
Daylight	<i>C. caeruleus</i>	0.93095	0.947	<b>1.05258</b>	<b>1.07667</b>
	<i>G. gallus</i>	0.64285	0.32915	0.80254	<b>1.06662</b>
Forest shade	<i>C. caeruleus</i>	0.82098	0.81856	0.97458	<b>1.00635</b>
	<i>G. gallus</i>	0.5896	0.31166	0.69908	0.96529

Comparisons were made using the blue tit (*Cyanistes caeruleus*, UV-type) and chicken (*Gallus gallus*, violet-type) cone sensitivities, and are based on mean values ( $n = 24$ ). Units are in just noticeable differences (JNDs). Bold indicates comparisons that exceed the threshold of discriminability (1 JND).



**Figure 2.** Survival curves of the three palatable facsimile phenotypes used in a preliminary control study. The three species vary in abundance and detectability. All of the facsimiles were attacked by predators at equal rates and shared equal survivorship ( $P > 0.5$ ; Cox proportional-hazards regression model).

determined parameters of Olsson et al. (2015), namely, a Weber fraction = 0.06 and relative abundances of cones (violet = 0.25,  $S = 0.5$ ,  $M = 1$ ,  $L = 1$ ). For the blue tit, we followed the work of Hart et al. (2000) and used a Weber fraction = 0.05 and relative abundances of cones (ultraviolet = 0.37,  $S = 0.7$ ,  $M = 0.99$ ,  $L = 1$ ). High light intensity and Endler's daylight or forest shade irradiance spectra (Endler 1993) were used; discriminability calculations are shown in Table 1.

Facsimile butterflies were printed on Whatman qualitative filter paper sheets (No. 1001–917) using an Epson Stylus Pro 4900 printer with Epson UltraChrome HDR ink. The filter paper produces reflectance spectra brightness similar to brightness in actual butterfly wings (Finkbeiner et al. 2012). Because some reflectance properties were difficult to reproduce with printed colors alone, Crayola® crayons (Easton, PA) were applied to portions of the printed wings to best match the actual butterfly wing spectra. The printed facsimiles were pasted onto cardstock backings with Krylon® Products Group spray adhesive (Cleveland, OH), then cut using a Brother ScanNCut machine (Brother International,

Bridgewater, NJ). Edges of the facsimile wings were dipped in wax as a seal, which allows facsimiles to be used multiple times in the field with minimal wear. Twist-ties were fixed onto facsimiles for attachment to foliage, then facsimiles were given plasticine abdomens (Newplast®) to allow detection of avian predator attacks.

### PRELIMINARY CONTROL STUDY

A preliminary predation study was carried out to determine an appropriate palatable control species to use for our main study, and to have a base predation rate on a known palatable species for future results comparisons. Three local, widespread palatable butterfly species were used: *Anartia fatima*, *Anartia jatrophae*, and *Junonia evarete* (Fig. 2). All three species are present at the La Selva Biological Reserve, but differ in abundance and detectability (pers. obs.). *Anartia fatima* was initially considered as our palatable control due to its high abundance, but its wing patterns closely resemble some *Adelpha* species. *Anartia jatrophae*, although much different in appearance, may be too detectable to predators because of the high background contrast generated by

its pearly white wings. *Junonia evarete* is less locally abundant, yet is a palatable group and like *Adelpha* has distribution across the tropics and temperate zones, making it a useful control for predation studies in multiple geographic areas. *Anartia fatima* has previously been used as a palatable control during in-cage bird predation studies on unpalatable *Heliconius* prey in Panama (Merrill et al. 2012), and all three species described above were readily eaten by jacamars during caged studies in Costa Rica (Chai 1986).

We constructed a total of 441 facsimile butterflies for this preliminary study: 147 of each butterfly species. The facsimiles were divided and set into 49 independent forest sites, each site containing three of each facsimile phenotype. The forest sites were separated by approximately 250 m to control for avian predator home range (Finkbeiner et al. 2012), which helps increase the number of bird populations sampled and to avoid disrupted results based on naïve bird behavior. Facsimiles within the forest sites were placed at least 4 m apart and in alternating order, and attached onto leaves or branches in appropriate resting positions (see Fig. S3) and at a height of  $\sim 1$  m from the ground. Facsimiles were exposed to predators for four days (96 h) and checked daily for bird attacks, which can be confirmed by the presence of beak imprints on the plasticine abdomen. We used a four-day predator exposure time period because previous work (Stobbe and Schafer 2008; Merrill et al. 2012; Finkbeiner et al. 2012, 2014; Seymoure and Aiello 2015; Dell'Aglio et al. 2016) shows this to be enough time for local predators in a home range to come in contact with (and attack) artificial butterfly facsimiles. Although we would benefit from an experiment examining long-term survival differences, facsimiles left exposed for too long risk being learned as artificial and inedible prey, which might skew further predation behavior; therefore we did not extend data collection longer than four days in each forest site. Examples of bird attacks on butterfly facsimiles are seen in Fig. S2, S3, Finkbeiner et al. (2012), and in Supporting Information in Finkbeiner et al. (2014). Attacks were modeled as dependent upon butterfly phenotype using a general linear model with Poisson distribution, including forest site as a random effect (R statistical software, R Development Core Team 2016). To examine butterfly survivorship curves across the four-day experimental period, we used a Cox proportional-hazards regression model for survival data in R (Fox and Weisberg 2011; Seymoure and Aiello 2015; R Development Core Team 2016 ["survival" package]).

#### MIMICRY PREDATION STUDY

Once our control species was determined, we used four different butterfly facsimile types to test whether locally convergent *Adelpha* species experience reduced predation rates compared to novel and known palatable (control) prey. The following butterfly species were used: (1) *Adelpha iphicles*, the presumed toxic

Rubiaceae-feeding model, (2) *A. serpa*, the presumed nontoxic, non-Rubiaceae-feeding mimic that shares a convergent wing pattern with *A. iphicles*, (3) *Adelpha leucophthalma*, an allopatric, nonlocal species possessing a wing pattern novel to avian predators, and (4) *J. evarete*, a known palatable butterfly species used as a control for observing a central predation rate (Figs. 1 and S2).

Five hundred individuals of each butterfly phenotype were used in this study, totaling 2000 facsimile butterflies. This sample size was chosen based on our expectations for lower predation rates overall, thus a power analysis was conducted using the low effect size of 0.075, general significance level of 0.05, and power of 0.80, which yielded a suggested total sample size of 1938. Following the experimental design of the preliminary control study, facsimiles were placed in 100 independent forest sites across the Reserve. Each forest site, separated by approximately 250 m, contained five of each of the four butterfly facsimiles set in alternating order. Facsimiles were placed at least 4 m from one another and  $\sim 1$  m above the ground on appropriate foliage. They were left in their respective sites for four days (96 h), and checked daily for avian attacks (see Fig. S2 for attack examples) then removed. The binomial response of attack (i.e., yes or no) was modeled as dependent upon butterfly facsimile type using a general linear model with a Poisson distribution and site as a random effect (R Development Core Team 2016). Following this analysis, we used a Cox proportional-hazards regression model in R (Fox and Weisberg 2011; Seymoure and Aiello 2015; "survival" package) to gain further information about differences in survival probabilities across the four-day experimental period. For both predation experiments, our specific methods and experimental design have been successful in previous studies testing avian predation on artificial butterfly prey in the tropics (Finkbeiner et al. 2012, 2014; Seymoure and Aiello 2015; Dell'Aglio et al. 2016).

## Results

### PREDATION RATES ACROSS LOCAL PALATABLE SPECIES

We conducted an initial study to determine predation rates between three local, palatable butterfly species using artificial butterfly facsimiles in predation studies. Using a total of 441 butterfly facsimiles, we recorded 40 bird attacks: 12 on *A. fatima* (8.2% attacked), 13 on *A. jatrophae* (8.8% attacked), and 15 on *J. evarete* (10.2% attacked). We found no statistical support for a difference in attack rates between the facsimile phenotypes; *A. fatima* was attacked at an equal rate to *A. jatrophae* (general linear model with Poisson distribution,  $z$ -value = 0.200, degrees of freedom [d.f.] = 146,  $P = 0.842$ ) and to *J. evarete* ( $z$ -value = 0.576, d.f. = 146,  $P = 0.565$ ); and *A. jatrophae* was attacked an equal rate to *J. evarete* ( $z$ -value = 0.378, d.f. = 146,  $P = 0.706$ ). We also examined whether survivorship curves between the butterflies differed

**Table 2.** Daily number of attacks (A) and survival rate (S) for each butterfly phenotype.

Butterfly phenotype	Day 1	Day 2	Day 3	Day 4	Total
<i>Junonia</i> (control)	A = 18 S = 0.964	A = 12 S = 0.976	A = 12 S = 0.976	A = 10 S = 0.98	A = 52 S = 0.896
<i>A. iphichlus</i>	A = 17 S = 0.966	A = 6 <sup>1,2</sup> S = 0.988	A = 5 <sup>1</sup> S = 0.99	A = 2 <sup>1,2</sup> S = 0.996	A = 30 <sup>2</sup> S = 0.94
<i>A. serpa</i>	A = 25 S = 0.95	A = 16 S = 0.968	A = 11 <sup>1</sup> S = 0.978	A = 4 <sup>1,2</sup> S = 0.992	A = 56 S = 0.888
<i>A. leucothalma</i>	A = 20 S = 0.96	A = 10 S = 0.98	A = 9 S = 0.982	A = 12 S = 0.976	A = 51 S = 0.898
Total	A = 80 S = 0.84	A = 44 <sup>1</sup> S = 0.912	A = 37 <sup>1</sup> S = 0.926	A = 28 <sup>1</sup> S = 0.944	A = 189 S = 0.906

<sup>1</sup>Where survival rate is significantly higher ( $p < 0.05$ ) than observations for that facsimile phenotype on the first day (across rows).

<sup>2</sup>Where survival rate is significantly higher than one or more facsimile phenotype on the day shown (down columns).

significantly with facsimile phenotype across the four-day experimental period, and found survivorship to be equal between butterfly facsimile phenotypes (Fig. 2). Similar to the overall predation results, *A. fatima* survivorship was no different than *A. jatrophae* survivorship (Cox proportional-hazards regression model,  $z$ -value = 0.201, d.f. = 2,  $P = 0.84$ ) or *J. evarete* survivorship ( $z$ -value = 0.582, d.f. = 2,  $P = 0.56$ ). *Anartia jatrophae* and *J. evarete* experienced equal survivorship probabilities ( $z$ -value = 0.382, d.f. = 2,  $P = 0.840$ ).

### PREDATION ON LOCALLY CONVERGENT PHENOTYPES

Our primary predation experiment tested whether *Adelpha* species with locally convergent wing patterns experience reduced predation rates compared to novel and known palatable butterfly prey. Two-thousand total artificial butterfly facsimiles were used in this study, which yielded 189 combined avian attacks: 52 on *Junonia* (10.4% attacked), 30 on *A. iphichlus* (6.0% attacked), 56 on *A. serpa* (11.2% attacked), and 51 on *A. leucothalma* (10.2% attacked, Fig. 1A). These attack rates are consistent with previous butterfly predation studies conducted in the tropics (Finkbeiner et al. 2014; Seymoure and Aiello 2015), and the *Junonia* attack rate is similar to our attack rate recorded on *J. evarete* during the preliminary study. Out of the 100 forest sites, 14 sites had wild *Adelpha* flying in the vicinity, as well as 15 sites having a jacamar or motmot predator present (among other insectivorous bird predators) at least once during the experimental period.

We found substantial evidence that attack rates vary between the facsimile phenotypes. Using a general linear model with Poisson distribution, we found that *A. iphichlus* was attacked significantly less than the other three facsimile phenotypes ( $z$ -value = 2.399, d.f. = 399,  $P = 0.0164$  vs *J. evarete*;  $z$ -value = 2.579, d.f. = 399,  $P = 0.0058$  vs *A. serpa*;  $z$ -value = 2.306, d.f. = 399,  $P = 0.0211$  vs *A. leucothalma*). The remaining facsimile phe-

notypes, *J. evarete*, *A. serpa*, and *A. leucothalma*, were attacked at equal rates (all  $P > 0.6$ ). An additional general linear model (R), using data in the form of proportions, produced identical results. Following this analysis, we examined whether survivorship curves between the butterflies differed significantly with phenotype using a Cox proportional-hazards regression model. Fifteen observations were automatically eliminated from the analysis (by the software) because no predation events were recorded for any butterflies across four days in those forest sites. The overall survivorship results from remaining observations parallel results from the predation model, showing *A. iphichlus* with the highest overall survivorship rate (94.0%) compared to the other facsimile butterfly phenotypes: *Junonia* (89.6% survivorship, significantly less than *A. iphichlus*,  $z$ -value = 2.655, d.f. = 3,  $P = 0.00792$ ), *A. serpa* (88.8% survivorship, significantly less than *A. iphichlus*,  $z$ -value = 2.358, d.f. = 3,  $P = 0.0184$ ), and *A. leucothalma* (89.8% survivorship, significantly less than *A. iphichlus*,  $z$ -value = 2.366, d.f. = 3,  $P = 0.018$ ; Fig. 1B). Survivorship curves did not differ between *A. serpa*, *A. leucothalma*, and *J. evarete* (all  $P > 0.7$ ).

Further comparisons on survival rate alone showed no difference in survivability for *Junonia* butterflies between days 1 and 4 ( $\chi^2 = 2.769$ , d.f. = 3,  $P = 0.429$ ), or for *A. leucothalma* butterflies between days 1 and 4 ( $\chi^2 = 5.863$ , d.f. = 3,  $P = 0.119$ ), implying their overall survival rate is constant. We note that survivorship is defined as the proportion of the original number of individuals in the cohort that are still alive/not attacked at the end of four days, whereas survival rate is the probability of surviving from one day to the next day. However, *A. iphichlus* and *A. serpa* individuals both had significantly higher survival rates toward the end of the four days of predator exposure compared to the beginning of the experiment, where *A. iphichlus* had greater survivability and was attacked less on days 2–4 compared to day 1 ( $\chi^2 = 17.2$ , d.f. = 3,  $P < 0.001$ ), and *A. serpa* had greater survivability (and

was attacked less) on day 4 compared to days 1–3 ( $\chi^2 = 16.714$ , d.f. = 3,  $P < 0.001$ ; Table 2). In support of this, an examination of survival rates on day 1 compared to survival average on days 2–4 shows that both *A. iphicles* and *A. serpa* have significantly lower survival rates on the first day than subsequent days (*A. serpa*:  $\chi^2 = 7.57$ , d.f. = 1,  $P = 0.00593$ ; *A. iphicles*:  $\chi^2 = 6.12$ , d.f. = 1,  $P = 0.0134$ ), but there is no difference in survival rates using the same comparisons with the two other butterfly species. These results suggest that predators avoid *A. iphicles* and *A. serpa* butterflies after longer exposure in the field, which might imply subtle support for our mimicry hypothesis; however, there are still at least twice as many attacks on *A. serpa* than *A. iphicles* on days 2–4 (Table 2) and *A. iphicles* has a significantly higher overall survivorship than *A. serpa* ( $z$ -value = 2.358, d.f. = 3,  $P = 0.0184$ ). We also found that across all four butterfly phenotypes, survival rate is equal on day 1 of predator exposure ( $\chi^2 = 1.9$ , d.f. = 3,  $P = 0.594$ ), but by day 4 *A. iphicles* has a higher survival rate than *Junonia* ( $\chi^2 = 5.3$ , d.f. = 1,  $P = 0.0209$ ) and *A. leucopthalma* ( $\chi^2 = 7.143$ , d.f. = 1,  $P = 0.00753$ ). *Adelpha serpa* also has slightly higher survival rate on day 4 compared to *A. leucopthalma* ( $\chi^2 = 4$ , d.f. = 1,  $P = 0.0455$ ), but not compared to *Junonia* ( $\chi^2 = 2.571$ , d.f. = 1,  $P = 0.1088$ ). Longer term survival projects with this system would be useful (e.g., Mallet and Barton 1989; Kapan 2001) to more closely examine a relationship between early loss (day 1) and long-term survival data on subsequent days, but long-term predation projects with artificial butterflies become complex with time as predators begin learning that facsimiles are not real prey.

## Discussion

### EVIDENCE FOR APOSEMATISM, BUT NOT BATESIAN MIMICRY

Based on mimicry theory, we predicted that *Adelpha* butterflies sharing locally convergent wing patterns would experience reduced predation rates compared to novel and known palatable prey. Contrary to this prediction, we found that unpalatable *A. iphicles* was attacked significantly less than palatable *A. serpa* (palatability assumptions based on larval host plant use; Aiello 1984; Ebel et al. 2015), suggesting that *A. serpa* does not benefit from sharing a convergent wing pattern with *A. iphicles*. Our observed attack rate on *A. iphicles* is closer to attack rates on unpalatable *Heliconius* butterflies at this field site (Finkbeiner et al. 2014), than to attack rates on known palatable butterflies (which was substantially higher). This implies bird predators perceive *A. iphicles* as an unpalatable species bearing a capture cost and therefore avoid them. These results provide the first evidence for protection of Rubiaceae-feeding *Adelpha*, suggesting this species is toxic and well defended (based on host plant use, Aiello 1984) and uses aposematic (warning) coloration to deter

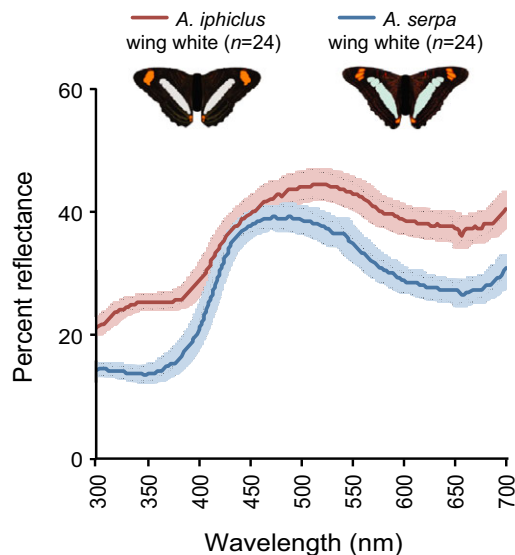
predators. Given the long-standing hypothesis that *Adelpha* are mimetic in nature, our new evidence for at least one toxic model (*A. iphicles*) supports the possibility that mimicry complexes and aposematism exist in this genus, which deserves further exploration in this field. However, we acknowledge that although *A. iphicles* experienced greater survivorship than *A. serpa* overall, these two similarly wing-patterned butterflies appear to suffer decreased relative predation in subsequent days during predator exposure. This result might imply a hint of Batesian mimicry, but the differences in predation between the two butterflies continued to remain statistically significant over the course of our experiment.

### PREDATORS DETECT DIFFERENCES BETWEEN CONVERGENT PATTERNS

Despite *A. iphicles* and *A. serpa* having nearly identical wing patterns, our results indicate that avian predators are capable of detecting subtle differences between the two and avoid facsimiles of the presumably toxic species, *A. iphicles*. Previous work has demonstrated that tropical bird predators pay close attention to color patterns on prey, especially with butterflies, and actively learn which prey can be eaten or should be avoided (Benson 1972; Chai 1986; Pinheiro 2003; Langham 2004; Ruxton et al. 2004). This implies mimicry and wing pattern convergence among locally sympatric butterfly populations is driven by natural selection through predation pressure, such that individuals possessing a novel or “incorrect” pattern face a greater risk of being detected and eaten (Kapan 2001). A previous study on butterfly wing pattern recognition by birds (particularly at this field station in Costa Rica) has noted the ability of birds to detect differences in placement of colors on butterfly prey wings, even when wing pattern remains the same (Finkbeiner et al. 2014). Observable differences between *A. iphicles* and *A. serpa* wing patterns might include the shape of the apical forewing orange patch, the thickness of the white band, or shape of the wings themselves.

Some of the major insectivorous predators at the La Selva Biological Station include near passerines such as jacamars and motmots, as well as passeriform tanagers and tyrant flycatchers (pers. obs.). Birds in general have sharp vision including a range of color discrimination abilities, with sensitivities in both the ultraviolet and violet wavelengths (spectral sensitivities summarized in Frentiu and Briscoe 2008; Ödeen and Håstad 2013). While at least one study found that birds do not differentially attack butterfly prey that differ in the ultraviolet (Finkbeiner et al. 2017), other studies have found that ultraviolet on lepidopteran wings may actually attract avian predators (Lyytinen et al. 2004). *Adelpha serpa* and *A. iphicles* color reflectance show the orange and brown spectra to be very similar (Fig. S4A and B), but the white scales on the wings have some differences in reflectance where *A. iphicles* is brighter overall (including in ultraviolet), and





**Figure 3.** Reflectance spectra of *Adelpha iphicles* and *Adelpha serpa* white wing bands. Shown are mean values (each  $n = 24$ ) with 95% CIs (shaded). *Adelpha iphicles* is brighter overall (including in UV), and *A. serpa* has more relative blue reflectance compared to *A. iphicles*.

*A. serpa* has more relative blue reflectance compared to *A. iphicles* (Fig. 3). This distinction between white band reflectances may be crucial for conspecific and mate recognition, and driven by sexual selection rather than predation as seen in other mimetic butterfly systems (Turner 1978; Su et al. 2015). Measurement of the blue- and long wavelength-absorbing rhodopsins of butterflies in the *Limnitis* lineage within *Adelpha* indicates that adult eyes contain photopigments with peak absorbances of  $\sim 430$  nm and 515–530 nm, respectively (Frentiu et al. 2007; Frentiu et al. 2015), which (together with the ultraviolet opsin) are in good locations to potentially permit the detection of differences between *A. iphicles* and *A. serpa* whites (Figs. 3 and S4). Additionally, based on our discriminability modeling results, it is likely avian predators are capable of detecting these spectral differences between the wing whites, and therefore able to recognize and avoid *A. iphicles*. However, under three of the four scenarios used in our avian vision model, the natural wing white comparisons done between physical butterfly specimens fall just under the discriminability threshold of 1 just noticeable difference, whereas three of the four comparisons for the artificial butterfly whites lie just above the threshold (Table 1), suggesting that the potential for Batesian mimicry to occur might be higher in nature than in the context of our experiment. To test this possibility, future experiments using an achromatic butterfly control to test for brightness alone (to determine whether color, instead of brightness, is a more effective stimulus) should be performed, especially because white coloration has been noted as an important mimetic feature

in other butterfly mimicry systems (Beccaloni 1997; Outomuro et al. 2016).

### IMPERFECT WING PATTERN CONVERGENCE

It has long been speculated that *A. serpa* is a Batesian mimic to *A. iphicles* (Aiello 1984), but our results show evidence that imperfections in convergent, potentially mimetic wing color patterns in *Adelpha* result in negative consequences. Imperfect mimicry, where the resemblance between models and mimics is inexact (Speed and Ruxton 2010), is successful for other mimicry systems such as hoverflies mimicking wasps (Dittrich et al. 1993), or nonvenomous snakes imprecisely mimicking venomous coral snakes (Kikuchi and Pfennig 2010). Although imperfect Batesian mimicry is not effective in our study, both frequency and capture costs (particularly distastefulness) play important roles in predator motivation to attack imperfectly mimetic Batesian prey (Lindström et al. 1997), and these two factors could potentially vary in the *Adelpha* system based on geographic location and host plant use scenario. Thus, in other geographic areas where both Rubiaceae specialist *A. iphicles* and generalist *A. serpa* co-occur, mimicry might function for *A. serpa* if there is a more favorable frequency of toxic *A. iphicles* models.

Our finding that the presumed mimic, *A. serpa*, receives no protection from predators begs the question: why converge on the same wing pattern? Several potential explanations exist including (1) *A. serpa* may have historically been unpalatable, such that *A. serpa* and *A. iphicles* were previously mimetic in a Müllerian context, but due to an ancestral host plant shift to a more tolerable plant, *A. serpa* may have lost its unpalatability; (2) there may be spatial and/or temporal variation in *A. serpa* palatability arising from polyphagous patterns of host plant use (palatability variation has been noted in the viceroy butterfly, suggesting the presence of both Müllerian and Batesian mimics (Ritland 1995)); (3) frequency-dependent dynamics may operate to cause seasonal variation in the perceived palatability of *A. serpa* to predators based on how common it is relative to *A. iphicles* (e.g., during our experimental period (dry season), *A. serpa* was more abundant than *A. iphicles*; C. Rush, pers. comm.); (4) the wing pattern represented by *A. serpa/A. iphicles* could provide an antipredatory benefit through disruptive coloration (see Seymoure and Aiello 2015) and/or may only be effective during flight (e.g., *Adelpha* are relatively unprofitable prey for birds due to their fast, unpredictable flight patterns and rapid escape ability (Mallet and Singer 1987; Pinheiro 1996)); (5) there may be thermoregulatory advantages to this wing pattern that are not relevant to mimicry, although this would likely be less important in lowland versus highland species (but see Srygley and Chai 1990); (6) there may be developmental constraints that repeatedly generate convergent expression patterns within *Adelpha* unrelated to mimicry; and, finally, (7) the

wing patterns on *A. serpa* may not be driven by natural selection at all but instead function to produce a signal to other butterflies, especially potential mates. Indeed, this latter hypothesis is intriguing because male *Adelpha* butterflies are territorial and individuals with similar phenotypes often defend perches at specific canopy heights (Willmott 2003), suggesting that female preference may be shaping both male behavior and color pattern. However, mate choice experiments will be necessary to resolve whether sexual selection is an important driver of wing phenotype evolution in *Adelpha*.

#### NOVEL AND LOCAL PALATABLE PREY ARE ATTACKED EQUALLY

An additional unexpected result from our study showed that novel, brightly colored allopatric *A. leucopthalma* butterflies were attacked equally compared to known palatable, dull *J. evarete* butterflies. *Adelpha leucopthalma* have large bright orange bands on the forewing and bright white spots on the hindwing (Fig. 1). Despite *A. leucopthalma*'s striking appearance, avian predators readily attacked this brightly colored novel butterfly phenotype, contrary to speculation that predators have an inherent avoidance of aposematic-like coloration (Smith 1975; Schuler and Hesse 1985; Rowe and Guilford 1996; Lindström et al. 1999; Ruxton et al. 2004). Here, birds treated aposematic novel prey and known palatable prey equally (also supported by Langham 2006 who found that older jacamars readily attack novel aposematic prey). The attack rates on *J. evarete* and *A. leucopthalma* parallel attack rates on novel allopatric *Heliconius* prey in a previous study at La Selva (Finkbeiner et al. 2014), and are similar to attack rates on *J. evarete*, *A. fatima*, and *A. jatrophae* in our prior control predation experiment. This provides evidence that some predators lump novel and palatable butterflies together as prey items that may be attacked without consequence, despite having different levels of prey knowledge, suggesting a high amount of risk behavior and prey testing by the predator. This is further supported by our survivorship results that showed no difference between *J. evarete* and *A. leucopthalma* survivorship rates across the four days, implying their overall survivorship declines at a constant rate.

Our preliminary experiment, which was carried out to identify a suitable control for the main predation experiment, showed equal predation and survivorship rates across three different local palatable species (*A. fatima*, *A. jatrophae*, and *J. evarete*) over the course of four days. The Three butterfly species vary in their coloration and abundance at the field station, but were still perceived as palatable prey whether previously encountered by predators or not. This again implies a level of risk behavior by bird predators that may vary in their experience and prior exposure to different prey types. *Anartia jatrophae*, despite bearing bright white coloration (making it presumably more detectable than the other two

species), was attacked at a similar rate to *A. fatima* and *J. evarete*. Collectively, these results suggest that high detectability in palatable prey does not necessarily entail lower survivorship compared to other palatable prey that are cryptic in appearance, but further quantitative studies using bird vision models to examine butterfly contrast against a foliage background are necessary to confirm this.

## Conclusions

Previous work has shown that rapid diversification in *Adelpha* is strongly associated with shifts in patterns of host plant specialization (Mullen et al. 2011; Ebel et al. 2015). In addition, phylogenetic evidence for convergent wing pattern evolution is widespread throughout the genus, often occurring between distantly related taxa feeding on both ancestral and derived host plant lineages (Ebel et al. 2015). Taken together with our new evidence for unpalatability to birds, these results suggest that natural selection may be acting in parallel at both the larval (host plant) and adult (wing color pattern) life-history stages to drive diversification in this lineage. Surprisingly, however, we found that *A. serpa* receives no protection from sharing a convergent wing appearance with the toxic Rubiaceae-feeding *A. iphiclus* model, suggesting that subtle imperfections in color (and pattern) among potentially mimetic butterfly prey are noticeable to predators in the tropics. Therefore, we conclude that complex dynamics, involving either geographic or temporal variation in palatability or other forms of selection (i.e., sexual selection), underlie convergent wing pattern evolution among *Adelpha* butterflies.

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#### DATA ARCHIVING

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Figure S1.** Specific examples of *Adelpha* wing pattern convergence. Red branches indicate taxa that share the *iphiclus/serpa* wing phenotype; an example of this phenotype is shown to the right of the red circle. Four major *Adelpha* mimicry types are displayed just below, where the number above each image indicates the number of species and subspecies with that pattern. From top left: *A. iphiclus iphiclus*, *A. naxia naxia*, *A. thesprotia*, *A. cocala cocala*, *A. justina justina*, *A. zina zina*, *A. salmoneus colada*, and *A. boreas boreas*. Figure modified with permission from Ebel et al. 2015.

**Figure S2.** Artificial butterfly facsimiles representing four major phenotypes: (A) *Adelpha iphiclus*, the presumed toxic Rubiaceae-feeding model; (B) *Adelpha serpa*, the presumed nontoxic, non-Rubiaceae-feeding mimic; (C) *Adelpha leucothalma*, an allopatric novel species; and (D) *Junonia evarete*, a local palatable species used as a control. Images on the right show evidence of bird attack.

**Figure S3.** Artificial butterfly facsimiles representing palatable species are placed in the forest for predation studies: (A) *Anartia fatima*, (B) *Anartia jatrophae*, and (C) *Junonia evarete*. Images on the right show evidence of bird attack.

**Figure S4.** Wing reflectance spectra for (A) *Adelpha iphiclus*, (B) *Adelpha serpa*, (C) *Adelpha leucothalma*, and (D) *Junonia evarete*.