



Warning signals are seductive: Relative contributions of color and pattern to predator avoidance and mate attraction in *Heliconius* butterflies

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Visual signaling in animals can serve many uses, including predator deterrence and mate attraction. In many cases, signals used to advertise unprofitability to predators are also used for intraspecific communication. Although aposematism and mate choice are significant forces driving the evolution of many animal phenotypes, the interplay between relevant visual signals remains little explored. Here, we address this question in the aposematic passion-vine butterfly *Heliconius erato* by using color- and pattern-manipulated models to test the contributions of different visual features to both mate choice and warning coloration. We found that the relative effectiveness of a model at escaping predation was correlated with its effectiveness at inducing mating behavior, and in both cases wing color was more predictive of presumptive fitness benefits than wing pattern. Overall, however, a combination of the natural (local) color and pattern was most successful for both predator deterrence and mate attraction. By exploring the relative contributions of color versus pattern composition in predation and mate preference studies, we have shown how both natural and sexual selection may work in parallel to drive the evolution of specific animal color patterns.

KEY WORDS: Aposematism, color pattern, mate recognition, predation, visual signals.

Animals display a variety of visual signals that serve multiple functions, including predator avoidance and mate signaling (Endler 1992). Sometimes, however, there may be interference between these signals. For instance, signals that aid in mate attraction frequently cause a higher risk of detection by predators (Endler 1980; Zuk and Kolluru 1998) whereas visual signals used to deter predation may also interfere with intraspecific communication and mate preference (Burns 1966; Estrada and Jiggins 2008; Nokelainen et al. 2012). Warning signaling—often referred to as aposematism—is a recurring phenomenon in the evolution of animal phenotypes where its principal function is to provide a signal advertising unprofitability to predators (Cott 1957; Guilford 1990; Ruxton et al. 2004). Warning signals are often communicated visually through conspicuous colors and patterns, and

although these signals are a significant force driving the evolution of many species, the relative importance of specific visual features contributing to aposematism remains little explored (Stevens 2007; Stevens and Ruxton 2012). Likewise, the evolutionary interplay between selection for warning signals and selection for other types of signals, specifically mating signals, also needs to be addressed in more depth. If the same visual signals have similar influence on predator avoidance and mate attraction, then there would be support for an honest signaling model (especially in the context of sexual signals) where information conveyed by an animal is useful to the receiver and can in turn increase its fitness (Zahavi 1975).

Neotropical passion-vine butterflies of the genus *Heliconius* have highly characteristic wing markings composed of vivid

colors and contrasting patterns. The butterflies are chemically defended by cyanogenic glycosides (Engler-Chaouat and Gilbert 2007), and thus represent a case of visually mediated aposematism. Previous studies have demonstrated the tendency of avian predators to attack unrecognized *Heliconius* morphs (Benson 1972; Mallet and Barton 1989; Kapan 2001; Langham 2004; Merrill et al. 2012), but it remains unknown what specific coloration features the predators use for prey recognition and how these features interact with the butterflies' mating signals. *Heliconius* butterflies have been shown to demonstrate assortative mating, or a nonrandom mating pattern where males and females prefer to mate with others of their own genotype and/or phenotype. Across the genus, assortative mating appears to be heavily influenced by wing color (Jiggins et al. 2001; Jiggins et al. 2004; Kronforst et al. 2007; Chamberlain et al. 2009; Melo et al. 2009). For butterflies and other animals, chromatic features (i.e., hue and saturation) are generally used for object identification and detection, whereas achromatic features (i.e., brightness, not hue and saturation) may play a significant role in detection under low-light conditions (Maier 1992; Vorobyev and Osorio 1998; Osorio et al. 1999; Kelber et al. 2003).

To determine to what extent warning signaling covaries with intraspecific signaling, we assessed the importance of color and pattern for both predation and mate selection in *Heliconius erato* butterflies. We define colors as consisting of hue, saturation, and brightness, and pattern as the size, shape, and location of patches (which can be chromatic, achromatic, or both), that are displayed on the wing. Here, we aim to answer four questions: (1) What role does color play in recognition of *H. erato* by experienced predators and conspecific males? (2) What role does pattern play in recognition of this species by experienced predators and conspecific males? (3) Is color more effective than pattern in recognition by experienced predators and conspecific males? (4) To what extent might color and pattern features have interfering, or parallel, effects on aposematism and mate attraction? We used color- and pattern-manipulated *H. erato petiverana* models in field studies to address these questions. We accounted for both chromatic and achromatic features by including achromatic models that lack color, making this one of the first studies to explicitly control for color alone. By exploring the contributions color and pattern for both mate and predator recognition, we were able to identify the significance of these visual features in the context of both natural and sexual selection.

Methods

FIELD SITES

All predation experiments were conducted at the Organization for Tropical Studies' La Selva Tropical Biological Station in Sarapiquí, Costa Rica. This work occurred in April and May of 2012,

during the end of the dry season into the beginning of the rainy season. All mate choice experiments were conducted in Panama at the Smithsonian Tropical Research Institute's insectary facilities in Gamboa. Butterflies were collected along Pipeline Road in the adjacent Soberanía National Park. Mate choice data were collected from June through October of 2012 during the rainy season. *Heliconius erato* butterflies in our Costa Rica and Panama field sites share the same wing phenotype.

PRODUCTION OF ARTIFICIAL BUTTERFLIES

We used artificial butterfly models to test the relative influence of color and pattern composition on mate choice and warning signaling, and to what degree the effects of these overlap. Artificial models were constructed according to Finkbeiner et al. (2012). Four model types were developed: a local phenotype model, a color-switched model (where the colors on the forewing and hindwing band were switched with one another), an achromatic model (no color: black, white, and grays only), and a nonlocal model which resembled *H. erato emma* but contained the same reds and yellows as local *H. erato petiverana* (Fig. 1). *Heliconius erato emma* is a South American morph that does not occur in the same geographic range as *H. erato petiverana* and therefore predators and other *Heliconius* should not have had prior exposure to this phenotype, although the ventral hindwing of *H. erato emma* may slightly resemble faint rays seen in certain morphs of *Heliconius doris*. Black pattern elements likely play an important role in receiver detection of species because black can provide high contrast against a foliage background (Stevens and Ruxton 2012). Because of this, black regions of model wings were kept black and not switched with any colored regions to promote equal rates of model detection by predators. Although our study would have benefited from using an achromatic nonlocal model type, we limited the number of prey options to four treatments to avoid confusing predators with too many choices, which has been suggested to be a problem in other predation studies (C. Jiggins, pers. comm.). As a follow-up experiment, however, we recorded predation on just two treatment types: achromatic models with the local pattern and achromatic models with a nonlocal pattern to confirm the importance of pattern composition alone, in the absence of color.

For predation studies, butterfly models were created to display the ventral side of *H. erato* wings because this area of the wing is exposed during rest. Models were designed to accommodate the avian visual system to minimize the ability of birds, the major predators of *Heliconius*, to distinguish between the color pattern stimuli presented by real butterflies versus experimental models. Tetrachromatic bird color-vision models, from two birds that differ in the spectral sensitivities of their short-wavelength-sensitive cone visual pigments, were used for discriminability modeling of color models—the UV-type (blue tit, *Parus caeruleus*) and violet-type (chicken, *Gallus gallus*) visual

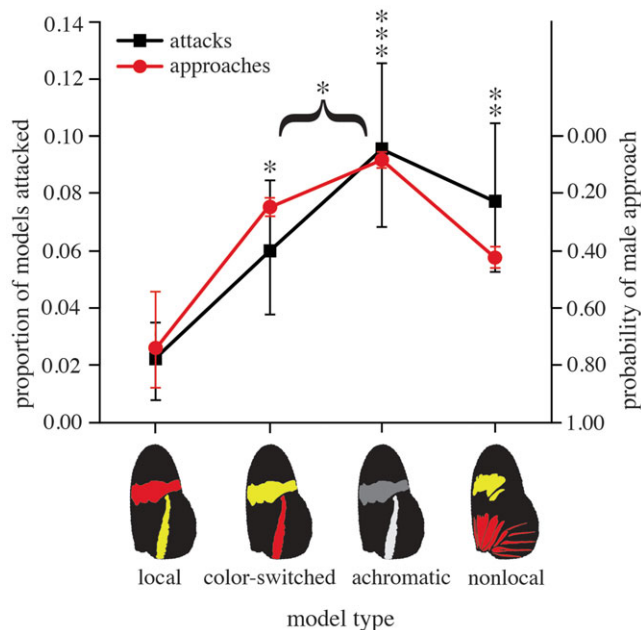


Figure 1. Color- and pattern-manipulated butterfly models experience different predation rates (left axis) and different probabilities of inducing premating approach behavior in male butterflies (right axis). There are four model types: a local *H. erato* type, a color-switched type, an achromatic type, and a nonlocal type. Error bars for the predation data include 95% CIs based on exact binomial distribution (Brown et al. 2002), and error bars for the mate preference data represent 95% credible intervals (Bayesian confidence intervals). Asterisks represent the *P*-values from pairwise comparisons between predation on the local model type and the three other model types, where **P* < 0.05, ***P* < 0.005, ****P* < 0.0001. All Bayes factors from approach probability comparisons show overwhelming evidence that the preference means differ between the model types (Bayes factors > 1.00×10^4).

systems (Vorobyev and Osorio 1998; Kelber et al. 2003). Previous quantitative models and experimental field studies suggest that the colors found on the artificial models and on the ventral side of *H. erato* are indiscriminable to avian predators of both visual types (Finkbeiner et al. 2012). For our achromatic models, we calculated the achromatic contrasts of their double cones for both the natural wing spectra and artificial gray spectra and selected the most similar grays for the artificial models (Bybee et al. 2012, eq. 2; Table S1).

For mate choice trials, the butterfly models presented both dorsal and ventral wing surfaces. Colors were selected for the ventral side of the artificial models as described above. To find appropriate dorsal colors to use for the models, spectral measurements were taken from the dorsal side of *H. erato petiverana* which consists of three major wing colors: red, yellow, and black. Measurements were taken using an Ocean Optics USB2000 fiber optic spectrometer (bifurcating fiber cable R400–7-UV–vis,

Ocean Optics, Winter Park, FL) with a deuterium–halogen tungsten lamp (DH-2000, Ocean Optics) used as a standardized light source. For every measurement, the axis of the illuminating and detecting fiber was placed in a probe holder at an elevation of 45 degrees to the plane of the wing, and pointed left with respect to the body axis. The spectrometer was calibrated during each use with a white spectralon standard (WS-1-SL, Labsphere, North Sutton, NH). We printed the artificial butterfly model wings on Whatman filter paper, which yields reflectance spectra close in brightness to actual butterfly wings, using an Epson Stylus Pro 4880 printer with UltraChrome K3 ink. A yellow pigment solution of 0.010 mg/μl 3-hydroxy-DL-kynurenine (3-OHK) in methanol was applied to the yellow bands on the ventral side, and a solution of 0.015 mg/μl 3-OHK in methanol was applied to the bands on the dorsal side to provide accurate UV reflectance. Because chromatic models contained methanol from the 3-OHK solutions, as a control methanol was applied to the area where the “yellow” band is located on achromatic models in case butterfly or predator response varied due to methanol odor. Appropriate colors were selected for models based on overall similarity to reflectance spectra of natural butterfly wings (Fig. S1). As an additional test to ensure the visual accuracy of the models, 5-min trials comparing approaches by randomly selected wild-caught *H. erato* males to *H. erato* models with artificial wings and *H. erato* models with real wings were conducted weekly, totaling 12 trials. Using a Wilcoxon signed-rank test with continuity correction, no difference was detected in approaches between real-wing models and artificial-wing models ($W = 27.5$, $P = 0.649$).

When considering possible differences between dorsal and ventral wing colors in *H. erato*, it is important to note that the shape of reflectance spectra for reds on both surfaces are nearly identical and show only slight variations in brightness. For yellows, the dorsal surface is brighter than the ventral surface. Nonetheless, we assume that this difference in brightness has little or no effect on a bird’s, or potential mate’s, ability to detect differences between colors, because chromatic features are more reliable signals under the variant illumination conditions of our experiment than are brightness features. Regardless of whether dorsal or ventral wings are displayed, avian predators and potential mates should have already learned both. Because the predation study focuses on the ventral wing side, and the mate choice study on the dorsal side, we interpret our results as assessing the potential for selection on both dorsal and ventral visual features.

PREDATION EXPERIMENTS

To test the relative influence of color and pattern composition on predator avoidance, we recorded predation attempts on models placed in the field. The models were fitted with plasticine abdomens and tied to branches with thread to represent

natural resting postures. We chose to use butterflies at rest because birds often attack butterflies in the morning hours while still at rest before foraging (Finkbeiner et al. 2012), and in other butterflies ventral wing characters appear to play a more important role in predator avoidance than do dorsal wing patterns (Oliver et al. 2009). Other studies investigating *Heliconius* predation have successfully used artificial models that display dorsal wing surfaces (Merrill et al. 2012), however we have observed that virtually all *H. erato* butterflies at rest in natural habitats hold their wings closed, thus exposing the ventral surface of the wing. We acknowledge that the actual butterflies' ventral wing bands appear slightly narrower than dorsal bands, and there is some evidence that the colored band elements on male *H. erato* are larger than those on females (Klein and Araújo 2013). In our artificial butterfly models, the dorsal and ventral wing bands are the same size.

Four individuals of each model type were randomly placed in 100 forest sites at our Costa Rica field location, totaling 1600 models used: 400 of each type (local, color-switched, achromatic, and nonlocal). Models were placed far enough apart so they were not within humanly visible range from one another (on average 5–10 m separated), and were positioned approximately 1.5 m above the ground, which is consistent with natural roosting heights of *H. erato* (Mallet and Gilbert 1995). Each forest site was at least 250 m apart to avoid overlap between predator home ranges (home range estimates are summarized in Finkbeiner et al. 2012) and no sites were used twice in the study to control for predator learning. Tree Tanglefoot® was applied to the base of plant stems containing artificial butterflies to prevent removal or attack of the models by small mandibulate arthropods. The models remained at their sites for a total of 96 h (four days), and each model was examined daily for evidence of predation. When a model was attacked, a substitute was placed in the same location, but any attacks on the substitutes were not included in the analysis. A model was determined attacked if the wings and abdomen had apparent beak marks and/or large indentations in the abdomen (see Fig. S2). If a model had more than one beak mark on it, this was counted only as a single attack. The binomial response of attack (i.e., yes or no) was modeled as dependent upon butterfly model type using a zero-inflated Poisson regression model, including sites as a random effect, with the “pscl” package (Zeileis et al. 2008; Jackman 2011) in R statistical software (R Development Core Team 2010). We later conducted a follow-up experiment in which we recorded predation on just two treatment types: 100 achromatic models with a nonlocal pattern, and 100 achromatic models with the local pattern, as a control for pattern in the absence of color. The models were placed in forest sites using the same methods described above, and data were analyzed using the aforementioned techniques.

MATE CHOICE EXPERIMENTS

To identify the relative contributions of color and pattern components in mate preference, we carried out mate choice experiments with wild-caught *H. erato* males using insectary facilities in Gamboa, Panama. We used males in this study because they are considered to be more active than females in insectary-based studies, and in nature females cannot accept a male until he has initially been attracted to and courted her. Although males and females of *H. erato* are sexually monomorphic in their color patterns, we do not rule out the possibility that males may have a biased selection toward a certain model type that could differ from female preference (see Kemp and Macedonia 2007). Prior to experimental use, the males were acclimated to the insectary environment for at least five days. Males were introduced individually into experimental cages (2 × 2 × 2 m) and presented with one of three pairs of the artificial butterfly models: local versus color-switched, local versus achromatic, and local versus nonlocal pattern. The local model represented the male's own color pattern. The artificial models, placed ~1 m apart, were fixed onto the ends of zip-ties attached to a PVC pipe suspended between two metal bars with monofilament. By tugging on the monofilament attached to another zip-tie in the center of the PVC pipe, the models could be manipulated to simulate the movement of butterflies in flight (see Video S1). The models imitated active flight behavior to appear realistic to males. In nature, males patrol for females in the home range and often approach to court females while females are in flight. Although the wing movements of the artificial models may vary from that of natural butterflies, our mechanical design made it possible to implement the most important control of having paired models displaying identical wing movements within trials.

Each individual male was presented with each of the three model pairs, in random order, three times. No males were presented with the same pair twice in one day. Mate choice trials with each pair lasted 5 min, beginning at the first sign of activity by the male. We randomized which models were placed on the north or south end of the flight simulator, and to control for males approaching models based on preference for a particular region of the cage, the models' placement was switched at 2.5 min. Individual males experienced nine 5-min trials—three 5-min trials with each pair. During trials, two variables were recorded: (1) approaches, which consisted of flight directed toward the model, and in which the male came within 20 cm of the model (see Video S2); and (2) courting attempts, which were classified as sustained hovering or circling behavior (lasting >1 s) around the model (see Video S3). Approach and courtship in *H. erato* are discrete, highly characteristic behaviors that are easy to identify and previous studies have used “approach” and “courtship” movements as a way to classify and measure butterfly response to artificial mates (Jiggins et al. 2001, 2004; Kronforst et al. 2006). All

courting attempts were also counted as approaches because a courting attempt is first initiated by an approach.

Mate preference data were analyzed using a hierarchical random effects Bayesian model for count data, which accounts for variation in both individual- and population-level preferences, as well as trial-by-trial variability. This statistical approach has been used in other recent studies to analyze count data in ecological and behavioral processes (e.g., Shiffirin et al. 2008; Fordyce et al. 2011; Lee 2011; Merrill et al. 2011a; Lee and Wagenmakers 2013). In our model, we denote by $d_{ijk}^{a,1}$ the count of approaches to the local model type for the i th butterfly on their k th trial in the j th condition, and $d_{ijk}^{a,n}$ for the count of approaches to the novel model type (color-switched, achromatic, or nonlocal). Similarly, there are $d_{ijk}^{c,1}$ and $d_{ijk}^{c,n}$ for the counts of courting attempts toward the local and novel model types, respectively. We assume there is an overall preference μ of choosing the local model type over any alternative novel model type. Each of the three novel model-type conditions is then assumed to have a preference for the local type that comes from a distribution centered around μ . These preferences are π_{cs} , π_{ac} , and π_{nl} for the specific color-switched, achromatic, and nonlocal conditions. Because π_j is the preference for the local model type, $1 - \pi_j$ is the preference for the novel model type. Specifically, $1 - \pi_{cs}$ is the preference for the color-switched type over the local type, $1 - \pi_{ac}$ is the preference for the achromatic type over the local type, and $1 - \pi_{nl}$ is the preference for the nonlocal type over the local type. There are assumed to be between-butterfly individual differences, drawn from a distribution with mean π_j , so that the i th butterfly on the j th condition has a latent preference given p_{ij} . There is also assumed to be between-trial variability for the same butterfly across the repeated trials, so that q_{ijk} denotes the latent preference of choosing the local model type for the i th butterfly on the k th trial in the j th condition. Finally, it is assumed that q_{ijk} is constant throughout trials, so the number of times the local model type was chosen $y_{ijk} = d_{ijk}^1$ follows a binomial distribution with this preference out of a total of $n_{ijk} = d_{ijk}^1 + d_{ijk}^n$ events. The overall preference μ and the condition-specific π_j preferences are the key parameters of interest.

We use beta distributions to model: (1) the condition-level variability that gives preferences of choosing the local model type over the three novel model types, (2) the group-level (population-level) variability that allows for individual differences between the butterflies within a condition, and (3) the trial-to-trial variability for each butterfly in each condition. The model is precisely illustrated by the graphical model shown in Figure 2, and additional details about the analysis are presented in Supporting Information. The population preference of choosing the local model type π_j for the j th condition is drawn from a beta distribution centered on the overall preference of choosing the local model type μ , with a precision λ^c , so that $\pi_j \sim \text{Beta}(\mu \lambda^c, (1 - \mu) \lambda^c)$. The

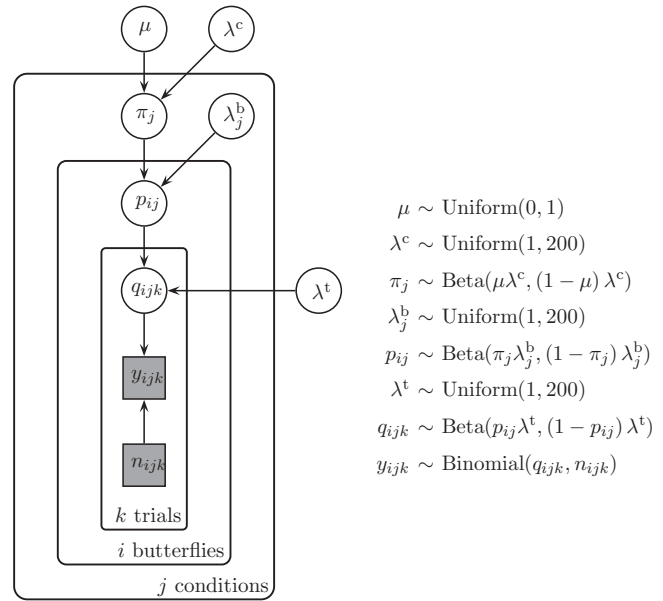


Figure 2. Graphical model of approach and courtship behavior across conditions (model type), assuming different mean preferences for each condition, and trial-by-trial variability for each butterfly in each condition. Continuous variables are shown as circular nodes, and discrete variables as square nodes. Observed variables are shaded whereas unobserved variables are not shaded. Plates are square boundaries that enclose subsets of the graph to indicate the subset has independent replications in the model. See Supporting Information for details.

preference for the i th butterfly in the j th condition is assumed to be drawn from a beta distribution with mean π_j and precision λ_j^b , so that $p_{ij} \sim \text{Beta}(\pi_j \lambda_j^b, (1 - \pi_j) \lambda_j^b)$. And finally, the preference and variability for the i th butterfly on its k th trial in the j th condition is assumed to be drawn from a beta distribution with mean p_{ij} and precision λ^t , so that $q_{ijk} \sim \text{Beta}(p_{ij} \lambda^t, (1 - p_{ij}) \lambda^t)$.

The model was implemented in JAGS software (Plummer 2003; for script see Supporting Information). The same model was applied independently to both the approach and courtship data. All of the analyses reported are based on six independent Markov chain Monte Carlo chains, each with 20,000 collected samples and 20,000 discarded burn-in samples. We evaluated standard measures of convergence and auto-correlation, including the \hat{R} statistic (Gelman 1996), to verify the samples as good approximations to the posterior distribution. To address whether pairs of the group mean preferences are the same or different, we used Bayes factors (Kass and Raftery 1995) estimated by the Savage–Dickey approximation method (Wagenmakers et al. 2010) to compare the prior and posterior density of the parameters. Details about robustness checks to examine the sensitivity of our results to quantitatively different ways of formalizing modeling assumptions are also presented in Supporting Information.

Results

PREDATION STUDY

To determine the relative influence of color and pattern composition on predator avoidance, we placed different model types of *H. erato* in forest sites. We observed the highest frequency of attacks on the achromatic model type, and the lowest frequency of attacks on the local model type (Fig. 1). Of 1600 artificial butterfly models, a total of 102 had evidence of bird attacks: nine local, 24 color-switched, 38 achromatic, and 31 nonlocal model types. Despite a low frequency of attacks overall, we found clear differences in attacks across all four model types. Visual inspection of the frequency of attacks along with our analysis indicates the largest difference in attacks was observed between the local and achromatic types (z value = 3.975, $P < 0.0001$), then between the local and nonlocal types (z value = 3.094, $P = 0.00197$), and finally between the local and color-switched types (z value = 2.500, $P = 0.0124$). We also found a difference in attacks between the color-switched and achromatic model types (z value = 2.266, $P = 0.0234$; Fig. 1). No statistically significant differences were detected in predation between the color-switched and nonlocal types, or between the achromatic and nonlocal types. For our follow-up experiment, which compared predation between achromatic models with a nonlocal pattern and achromatic models with the local pattern, we recorded nine attacks on the nonlocal pattern and three attacks on the local pattern, out of 100 models of each type. No statistically significant difference was detected (z value = -1.642 , $P = 0.101$).

MATE CHOICE STUDY

To assess the roles of color and pattern components in inducing mating-related behaviors, we recorded the responses of wild-caught *H. erato* males when presented with a series of different artificial butterfly models. Overall, we recorded 2224 approaches and 772 courtship attempts from 51 unique males during 438 5-min trials. Forty-seven out of the 51 males completed all nine 5-min trials (three trials with each pair), whereas three males only completed one set of three trials and one male completed two sets of the three trials. The data from those males were included in the analysis because each set of these trials still consisted of a test with all three pairs. The posterior means of the probability, or preferences, of males approaching and directing courtship attempts at the local model type (overall μ preference), and at the color-switched, achromatic, and nonlocal model types (condition-specific $1 - \pi_j$ preferences: $1 - \pi_{cs}$, $1 - \pi_{ac}$, and $1 - \pi_{nl}$), are presented in Table 1, along with their corresponding 95% credible intervals. Violin plots (Hintze and Nelson 1998) representing the posterior distributions of approach and courtship data, as well as figures showing the Savage–Dickey estimates, are presented in Supporting Information.

Our results show that males preferentially approached and courted conspecific (local-pattern) models more than any other model type (Fig. 1; Table 1). In addition, the evidence strongly suggests that approach and courtship preference means differ between the three novel model types. This evidence is based on extremely high Bayes factors and nonoverlapping posterior distributions in the estimation (see Supporting Information for interpretation details). Males showed a higher preference for approaching the nonlocal type than the color-switched type (Bayes factor = 2.01×10^4 in favor of the two preferences being different). Males also showed a higher preference for approaching the nonlocal model type than the achromatic type (Bayes factor = 1.75×10^4 in favor of the two preferences being different), and the color-switched model types were preferred and approached more than the achromatic ones (Bayes factor = 1.09×10^4 in favor of the two preferences being different). The Bayes factor of 1.09×10^4 , for instance, indicates that the data are 1.09×10^4 times more likely to have arisen if the group means for the color-switched and achromatic model types are different, rather than if they are the same. We found similar results with respect to courting attempts: males showed a higher preference for courting models of the nonlocal type than the color-switched type (Bayes factor = 1.67×10^4). They also preferred to court models of the nonlocal type more than the achromatic type (Bayes factor = 2.69×10^4), and the color-switched model types were preferred and courted more than the achromatic model types (Bayes factor = 1.02×10^4). In summary, males predominantly preferred to approach and court their own type, followed by (in consecutive order) the nonlocal type, color-switched type, and finally the achromatic type.

Discussion

RELATIVE EFFECTS OF COLOR AND PATTERN ON PREDATION RATES

In this study, we tested the relative influence of color and pattern features in both predator avoidance and mate preference. We found that wing color and pattern composition appear to play roles in both cases, although color likely has a greater influence than pattern on predator and conspecific recognition. From our predation results we conclude that color alone acts as a successful aposematic signal in *Heliconius* butterflies because achromatic models (possessing the same pattern, but no color) were attacked significantly more than the local model (Fig. 1).

We also found that pattern appears to play some role in aposematic signaling in *H. erato*—although the evidence for this in our own study has some weakness. Specifically, we found that nonlocal models possessing a novel pattern, but the same colors as the local model type, were attacked significantly more often than the local model. However, because the placement of colors

Table 1. Occurrences and probabilities of approach and courtship between model types.

(a) Male display	Color-switched:local	Achromatic:local	Nonlocal:local	
Ratios of <i>H. erato</i> approach	181:558	51:566	369:499	
Ratios of <i>H. erato</i> courtship	46:216	6:221	94:189	
(b) Male display	Local μ	Color-switched $1 - \pi_{cs}$	Achromatic $1 - \pi_{ac}$	Nonlocal $1 - \pi_{nl}$
Probability of <i>H. erato</i> approach	0.739	0.243	0.086	0.422
(Credible intervals)	(0.541, 0.881)	(0.212, 0.276)	(0.063, 0.110)	(0.387, 0.457)
Probability of <i>H. erato</i> courtship	0.814	0.173	0.034	0.324
(Credible intervals)	(0.606, 0.944)	(0.128, 0.223)	(0.010, 0.062)	(0.270, 0.382)

Mate choice results shown as (a) the ratios of approach and courtship occurrences for male *H. erato* butterflies during paired trials with the local model type and the color-switched, achromatic, and nonlocal type, respectively; and (b) probabilities of approach and courtship, estimated using a hierarchical Bayesian framework, representing the overall preference μ of choosing the local model type over all other novel model types, as well as the group preferences $1 - \pi_j$ of choosing the novel model types. The 95% credible intervals (Bayesian confidence intervals) are shown in parentheses. The probabilities and credible intervals are graphed in Figure 1.

(e.g., red vs. yellow on the forewing) is not the same between these two models, hue and brightness differences between yellow and red could also account for the differences in attack rate. A more informative comparison for the possible effect of pattern is the comparison between the color-switched and the nonlocal model because in this case the placement of colors on the wing is similar so there are no brightness or hue differences. In this controlled comparison for pattern, no significant difference was detected in attacks between the color-switched model and nonlocal model. Furthermore, our follow-up experiment detected no significant difference in attacks between achromatic local and achromatic nonlocal patterned models, suggesting that in the absence of color, particular patterns by themselves appear to have little specific effect as warning signals. Nonetheless, a combination of the appropriate colors and patterns is likely important for optimal predator deterrence in *Heliconius*.

Our findings are consistent with previous work by Aronsson and Gamberale-Stille (2008) that found avian predators primarily attend to color, rather than pattern, when learning aposematic visual signals. Studies focusing on the importance of visual signals in predator avoidance of other aposematic animals provide evidence that a bright color alone provides protection (Ruxton et al. 2004), but in some snakes, the correct combination of colors is fundamental for predator recognition and avoidance (Brodie 1993). Similar studies have shown that dragonflies are more likely to avoid wasp-like stripes rather than uniform black or yellow, indicating the influence of pattern on their foraging decisions (Kauppinen and Mappes 2003), and with inexperienced chicks, striped patterns can increase avoidance when coupled with colors that are not typically associated with a cost (Hauglund et al. 2006). Evidence that predators avoid aposematic colors more readily than a particular aposematic pattern could be due to the fact that predators that target fast-moving prey may have difficulty identifying

precise patterns during prey movement, whereas detecting colors would be much easier. Perhaps this is why aposematic prey often have markings comprised of repeated pattern elements which could improve the likelihood of detection (Stevens and Ruxton 2012).

RELATIVE EFFECTS OF COLOR AND PATTERN ON INDUCING MATING BEHAVIOR

Our mate preference experiments had similar outcomes to the predation study with respect to color. We found that all colored models were considerably more successful at triggering mating-associated behaviors than the achromatic model. This evidence that males are highly responsive to chromatic features is consistent with previous findings that *H. erato* have excellent color vision in the long-wavelength range in the context of feeding (Zaccardi et al. 2006). Our new results provide evidence that color discrimination in the long-wavelength range also matters for mating behavior, although further experiments would be required to confirm this.

With respect to pattern, we found that the local model type was the most effective at inducing mating behavior in males. Unlike the predation experiments, however, we found strong evidence for a difference in the preference means of approach and courtship between the nonlocal and color-switched models. The nonlocal pattern was preferred more, suggesting that pattern may play a more significant role in mate preference than in predation. Although both the color-switched and nonlocal model types presented yellow bands on the forewing, the nonlocal model had a greater surface area of red on the hindwing than the color-switched model. Previous studies have shown that male *Heliconius* butterflies are strongly attracted to the color found on the forewing band (Kronforst et al. 2006; Merrill et al. 2011b), which in this case is red, so it is possible this preference is in part due to a greater area

of red on the wings. An alternative explanation could be that there is some inherent preference for the rayed pattern in the nonlocal model that is shared across different *H. erato* races.

It is important to note that although we provide evidence that color plays a proximate role in conspecific recognition and mate preference, it does not necessarily mean that it is a product of sexual selection (for a discussion of these issues, see Mendelson and Shaw 2012); although we do speculate that this is the case. When considering the evolution of coloration, a key question (also raised by Kemp and Macedonia 2007 and Kunte 2009) is whether male preference leads to significant variation in female mating success, which would in turn lead to selection on female coloration. Nielson and Watt (2000) proposed that females that are approached less frequently by males suffer a reduction in fitness because they spend more of their time in a nonfertile state. This effect could be additionally amplified in *H. erato* because older females—virgin or not—attract fewer courtship attempts by males (Klein and Araújo 2010), so the longer a female waits to mate, the lower her chance of mating becomes. It has also been proposed that females that settle to mate with fewer, older, and/or smaller males should receive reduced nutritional benefits from poorer quality spermatophores (e.g., Rutowski et al. 1987). Again, this effect could be amplified in *H. erato* due to the limited number of matings wild females experience owing to their postmating male “antiaphrodisiac” pheromones (Gilbert 1976; Estrada et al. 2011). In addition to these fitness effects, genetic work has shown that genes causing wing pattern variation have the same effects on both sexes (Papa et al. 2013). Thus, selection on wing patterns in one sex would be expected to affect the wing patterns of both sexes. In sum, although little work has been done to empirically determine the fitness effects of male bias in *Heliconius*, precedents in other butterfly systems make it reasonable to speculate that male preference should lead to selection on coloration in females.

Many *Heliconius* mate preference studies have focused on species from the polyandrous “adult mating” *melpomene-cydno* clade, however our study presents some of the first mate preference data using *H. erato* as the study species. We have shown here that males exhibit strong color pattern-based preferences toward conspecific phenotypes (suggesting assortative mating in this species), and they actively approach and court artificial models despite being members of the *Heliconius* “pupal mating” clade, in which females are typically monandrous and males are not expected to be vigorous courters (Gilbert 1976, 1991; Deinert et al. 1994; McMillan et al. 1997; Estrada et al. 2011; Walters et al. 2012). These behavioral observations also suggest that *H. erato* may commonly mate as adults, although more rigorous field studies need to be done to confirm this (but see Klein and Araújo 2010 for information about adult courtship behavior in *H. erato*).

COLORS FEATURES ARE BETTER PREDICTORS OF FITNESS-RELATED EFFECTS THAN PATTERN FEATURES

As described above, our predation and mate preference studies suggest that color is a more broadly effective visual signal than pattern. Namely, the achromatic model was attacked by predators more than any other model, and also had the lowest probability of inducing mating behavior in male butterflies. Even further, the fact that males responded more to the nonlocal type than to the color-switched type shows that for any given pattern, color matters for mate preference. A similar study by Kronforst et al. (2006) showed that yellow male *H. cydno* have a higher probability of courting their own yellow type than the white type, even when pattern remains the same. With respect to pattern, we found some evidence that pattern matters for male preference as have previous studies (Chamberlain et al. 2009). In the lycaenid butterfly *Lycaeides idas*, females with reduced ventral wing pattern features were less preferred than females with unmanipulated patterns (Fordyce et al. 2002). Although a combination of the correct colors and pattern is important for both warning coloration and mate attraction in *H. erato*, we conclude that color likely contributes more to overall signal effectiveness in both circumstances.

WARNING COLORATION AND MATE CHOICE SIGNALS WORK IN PARALLEL

One of the most interesting findings from our study is that visual features used for both predator avoidance and mate attraction produce similar effects on fitness-related traits. The results from both sets of experiments show consistent overlap between the model treatments attacked most by predators and those least effective at inducing male mating behavior (Fig. 1). We acknowledge, however, that although our results suggest mate choice and predation will produce selection for similar colors and patterns, we have not evaluated in detail the relative strength of selection by each of these two pressures. It is possible one selective force may influence evolution by the other if substantial imbalance exists between them (for a review on this topic, see Kunte 2009); but confirming this will require further work within this system.

In *H. erato*, the phenotype most effective in preventing predator attack is selected most by males, suggesting its appearance provides a signal to potential mates demonstrating greater survival probability for both itself and offspring. This implies an honest signaling model where information communicated by an animal is useful to the receiver and can in turn increase its fitness (Zahavi 1975). Therefore, males should invest greater energy in courting females that display their same phenotype. A similar situation has also been described in the poison-dart frog *Oophaga pumilio* where aposematic coloration also serves as an attractive signal to mates (Maan and Cummings 2008). This positive

interaction between aposematism and mate choice indicates cooperation between visual signals that benefit individual fitness. By identifying the contributions of color versus pattern in predation and mate preference studies, we have shown how both natural and sexual selection may work together to reinforce the evolution of coordinated suites of visual signals.

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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:

Supporting Information Modeling Details: Modeling details for hierarchical Bayesian analysis.

Table S1. Achromatic contrasts (using double cones) for both the natural wing spectra and artificial wing gray spectra.

Figure S1. Reflectance spectra of the natural and artificial (paper) model dorsal wing colors used in mate preference studies.

Figure S2. Evidence of predation attempts on a color-switched (a) and nonlocal (b) butterfly model.

Video S1. Example of flight simulator in use with two artificial butterfly models and a wild-caught male *H. erato* approaching from the left side.

Video S2. Example of approaches directed toward an artificial model of *H. erato* (with the local appearance) by a wild-caught male.

Video S3. Example of courtship behavior directed toward an artificial model of *H. erato* (with the local appearance) by a wild-caught male.