

Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog

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It is commonly assumed that natural selection imposed by predators is the prevailing force driving the evolution of aposematic traits. Here, we demonstrate that aposematic signals are shaped by sexual selection as well. We evaluated sexual selection for coloration brightness in populations of the poison frog *Oophaga [Dendrobates] pumilio* in Panama's Bocas del Toro archipelago. We assessed female preferences for brighter males by manipulating the perceived brightness of spectrally matched males in two-way choice experiments. We found strong female preferences for bright males in two island populations and weaker or ambiguous preferences in females from mainland populations. Spectral reflectance measurements, coupled with an *O. pumilio*-specific visual processing model, showed that *O. pumilio* coloration was significantly brighter in island than in mainland morphs. In one of the island populations (Isla Solarte), males were significantly more brightly colored than females. Taken together, these results provide evidence for directional sexual selection on aposematic coloration and document sexual dimorphism in vertebrate warning coloration. Although aposematic signals have long been upheld as exemplars of natural selection, our results show that sexual selection should not be ignored in studies of aposematic evolution.

aposematism | color | dendrobates | divergence | polymorphism

Aposematic traits evolve under natural selection to warn predators of prey unprofitability. Because predators are expected to exert stabilizing selection on aposematic coloration, the variability of aposematic traits in nature remains a biological enigma. Aposematic signal divergence may be driven by spatially variable or frequency-dependent predation (1–4), but the potential role of sexual selection is rarely addressed. It stands to reason however, that sexual selection affects aposematic evolution, because aposematic traits can also function in mate recognition (5, 6). When ecologically important traits are under sexual selection by female choice, they may become sexually dimorphic as a response to the dual selection pressures on one sex versus a single selection pressure on the other (7). Here, we explore whether sexual selection can influence the evolution of warning signals in a polymorphic poison frog, *Oophaga [Dendrobates] pumilio* (8). We test for directional selection by female choice for males with brighter aposematic signals and examine sexual dimorphism in this trait.

Throughout most of its geographic range, across the mainlands of Nicaragua to Panama, *O. pumilio* coloration is red dorsally and ventrally with dark blue arms and legs (9). However, ≈15 strikingly different phenotypes, spanning the full range of the visual spectrum (10–12), inhabit peninsular and island areas in the Bocas del Toro archipelago in northwestern Panama. These populations vary in both dorsal and ventral coloration, including hue (the specific waveband of reflectance), brightness (the quantity of light reflected), and spotting pattern. Relatively recent geographical isolation (<10,000 years) (13) and incomplete lineage sorting imply that genetic drift alone cannot explain the observed variation (15, 16). Instead, island populations may experience different selective regimes from those prevailing on the mainland: strong directional selection and/or release from stabilizing selection, possibly interacting with drift (17–19).

Although *O. pumilio* are toxic (10, 20), and their color patterns likely deter predators (21, 22), laboratory experiments show that coloration also plays a role in female mate choice (6, 23, 24). Sexual selection could be important in *O. pumilio*, because females provide their tadpoles with nutritional eggs for several weeks, whereas male care is limited to guarding and moistening the eggs for a few days until hatching (15, 25). This asymmetry in parental investment is consistent with strong female mate discrimination (26).

In *O. pumilio*, signatures of sexual selection on coloration have been identified at the interpopulation level, because females generally prefer native over foreign males (6, 23, 24). In this study, we explore whether male coloration could also be subject to sexual selection within populations. Recently, we found that *O. pumilio* females may tolerate or even prefer males that deviate from the average phenotype in their native population (24). Such preferences can result in directional selection on male traits, potentially contributing to population divergence (27).

We focus on one potential target of directional sexual selection in this system: aposematic brightness. Many of the *O. pumilio* morphs that inhabit the islands of the Bocas del Toro area have brighter coloration than the red/blue mainland morph (see *Results*), suggesting a role for selection on brightness. Moreover, color pattern brightness is a prevalent component of aposematic conspicuousness that has been shown to increase learning and retention by predators (28). It is also targeted by sexual selection: Female preferences for louder, brighter, bigger males are widespread in the animal kingdom (29, 30). Importantly, brightness and hue (color) are interdependent features of a visual signal, and changes in signal brightness may lead to changes in signal hue. Hence, studying the selective pressures affecting signal brightness in this system may provide insight into the mechanism of color diversification as well.

Here, we evaluate female preferences for brighter males, by manipulating male apparent brightness in laboratory mate choice trials. Further, if sexual selection for brighter coloration is a significant selective agent in the *O. pumilio* system, it may lead to brighter coloration in males than in females. We therefore use reflectance spectrophotometry and visual modeling to quantify patterns of sexual dimorphism. Finally, interruption of gene flow and release from selective constraints that prevail on the mainland may lead to stronger signatures of selection in island versus mainland populations (31, 32). Therefore, we study four geographically distinct populations of *O. pumilio* that represent a broad range of hues and brightness levels: the ancestral red/blue mainland morph and three derived nonmainland populations (orange, green, blue; Fig. 1A).

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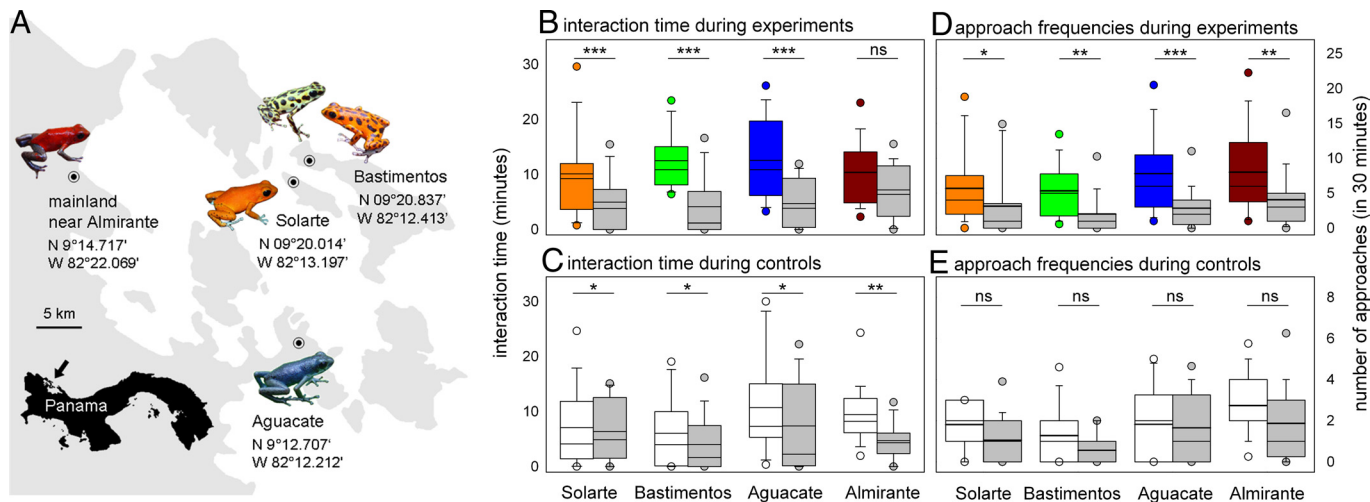


Fig. 1. Study populations and mate choice results. (A) The four study populations that represent the diversity of hues observed in *O. pumilio*. (B–E) Results of the mate choice experiments. (B) Experiments: female interaction time with the brightly illuminated male (colored boxes) and the dark illuminated male (gray boxes). (C) Controls: interaction time near the brightly illuminated compartment (open boxes) and the dark illuminated compartment (gray boxes). (D) Experiments: frequency of approaches toward the brightly illuminated male (colored boxes) and the dark illuminated male (gray boxes). (E) Controls: frequency of approaches toward the bright compartment (open boxes) and the dark compartment (gray boxes). Boxes indicate 25–75 percentiles intercepted by the median (thin line) and mean (thick line); error bars indicate 10–90 percentiles, symbols are 5–95 percentiles. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Results

Female Preference for Brighter Males. In three of four populations, females spent significantly more time interacting with the more brightly illuminated male (Fig. 1B and supporting information (SI) Table S1). In control trials without males, females from all four populations spent more time near the brighter compartment as well (Fig. 1C), but females from the islands of Solarte and Bastimentos showed significantly stronger preferences in experiments than in controls ($\chi^2 > 4.78$, $df = 1$, $P < 0.029$). Taking all populations together, there was a significant preference difference between experiments and controls ($\chi^2 = 5.49$, $df = 1$, $P = 0.0192$), with females spending significantly more time interacting with brighter males than they did in the vicinity of brighter compartments without males.

We also found that females from all four populations approached the more brightly illuminated males more frequently ($\chi^2 > 3.94$, $df = 1$, $P < 0.047$; Fig. 1D and E, Table S1), whereas such preferences were absent during control trials. Approach frequencies in control trials were low, however, and the difference between experiments and controls was not significant for

any of the four populations, nor when taking all populations together ($\chi^2 = 2.29$, $df = 1$, $P = 0.13$).

Population Differences in Brightness (Male and Female Reflectances Combined). There were highly significant differences in dorsal brightness across the four study populations for both measures of brightness [total reflectance flux ($\Sigma R(\lambda)$): $F_{4,220} = 69.028$, $P < 0.0001$; visual estimates of brightness contrast with background vegetation (ΔL): $F_{4,220} = 89.18$, $P < 0.0001$]. Aguacate frogs were darkest, followed by Almirante (mainland) and the two island populations from Solarte and Bastimentos, with Bastimentos green frogs showing the highest brightness (Fig. 2). Pairwise comparisons [Tukey's honestly significant difference (HSD)] showed that all populations differed significantly from each other in both brightness measures (all $P < 0.01$) except for Bastimentos orange frogs that did not differ from Almirante in total reflectance flux ($P = 0.064$) and had similar brightness contrast as Solarte ($P = 0.93$).

Populations also differed significantly in ventral brightness [$\Sigma R(\lambda)$: $F_{3,210} = 164.61$, $P < 0.0001$; ΔL : $F_{3,210} = 201.69$, $P < 0.0001$]: lowest in mainland frogs from Almirante, followed

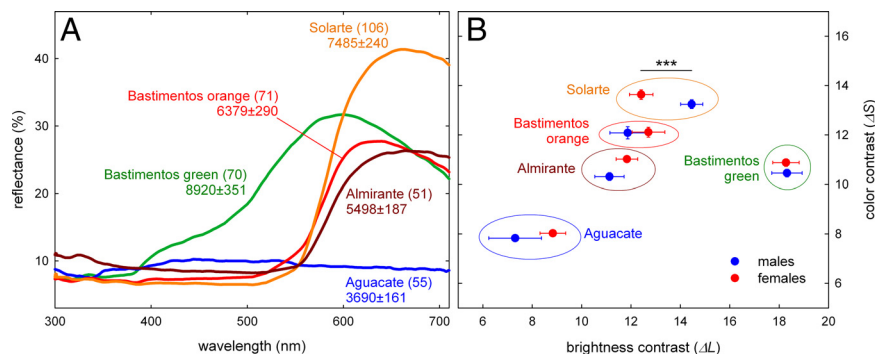


Fig. 2. Dorsal coloration in the four study populations of *O. pumilio* (with two color morphs for the Bastimentos population). (A) Average reflectance spectra (males and females combined). Numbers indicate sample size (in brackets) and mean \pm SE total reflectance flux [$\Sigma R(\lambda)$]. (B) Estimated brightness (ΔL) and color (ΔS) contrast for the *O. pumilio* visual system generated by male and female dorsal reflectance spectra viewed in a natural light environment. Solarte is the only population in which contrast differences between males and females exceed the detectability threshold (mean $\Delta L_m - \text{mean } \Delta L_f = 2.05$). See also Figs. S1 and S2.

by Solarte, Aguacate, and Bastimentos (Fig. S3). Pairwise comparisons (Tukey's HSD) showed that Bastimentos frogs had significantly brighter ventral coloration than the other three populations in both measures (all $P < 0.0001$; green and orange Bastimentos frogs did not differ in any measure of ventral coloration, all $P > 0.9$, and are treated as one population). Almirante, Aguacate, and Solarte did not differ from each other ($P > 0.19$) except for a significantly higher ventral brightness contrast of Aguacate frogs compared with Almirante ($P < 0.00044$).

Thus, all three (pen)insular populations had brighter coloration than the mainland Almirante population on either dorsum (Solarte), venter (Aguacate), or both (Bastimentos).

Within-Population Differences: Sexual Dimorphism. In the island population of Solarte, males were significantly brighter than females (total reflectance flux, $\Sigma R(\lambda)$): dorsal: males $7,238 \pm 280$, females $6,187 \pm 257$, $F_{1,103} = 11.82$, $P = 0.00085$; ventral: males $8,379 \pm 328$, females $7,674 \pm 232$, $F_{1,102} = 4.49$, $P = 0.037$). This generated significant differences in brightness contrast that should be perceptible by conspecifics [i.e., just noticeable difference (jnd) > 1 : dorsal mean $\Delta L_m - \text{mean } \Delta L_f = 14.46 \pm 0.44 - 12.41 \pm 0.47 = 2.05$, $F_{1,103} = 13.76$, $P = 0.00034$, Fig. 2B; ventral mean $\Delta L_m - \text{mean } \Delta L_f = 16.08 \pm 0.47 - 14.96 \pm 0.35 = 1.13$, $F_{1,102} = 11.99$, $P = 0.00078$, Fig. S3B]. There was no brightness dimorphism in the other three populations [$\Sigma R(\lambda)$ and ΔL : all $P > 0.16$].

We found one instance of possibly perceptible dimorphism (jnd > 1.0) in color contrast: Almirante females had higher ventral color contrast than males (mean $\Delta S_m - \text{mean } \Delta S_f = 1.05$, $t_{44} = 2.77$, $P = 0.0081$).

Discussion

The spectacular color polymorphism of *O. pumilio* has fascinated biologists for decades (e.g., refs. 10, 15, and 33), but its evolutionary underpinnings have remained unclear. Previous studies have demonstrated that *O. pumilio* female mate choice is influenced by between-population differences in coloration (hue and brightness combined) (6, 23, 24), suggesting that female choice helps to maintain existing diversity. Here, we present observations and experiments that indicate how female choice could contribute to evolutionary change in coloration traits: Female preferences exert directional selection on male coloration brightness within populations. By documenting female preferences for brighter males as well as sexual dimorphism in brightness within populations, our study provides the first evidence for directional sexual selection on an aposematic signal.

Our mate choice experiments revealed that females in populations with the brightest dorsal reflectances consistently prefer brighter males (Solarte and Bastimentos islands). In contrast, females from mainland populations with darker coloration showed moderate or ambiguous preferences (Almirante mainland and Aguacate peninsula). Brighter coloration (and stronger female preferences for brightness) in island populations could reflect a different natural selection regime. For example, differences in predator communities or alkaloid availability may entail changes in the direction and strength of natural selection, affecting both color phenotypes and mate preferences (20, 34–36).

In one of the brighter populations, Solarte, males were significantly brighter than females and showed stronger contrast to background vegetation. Importantly, this difference is not only discernable to the *O. pumilio* eye in its natural light environment (see ΔL estimates in Fig. 2), but is also likely to be detected by any predator with long-wave visual sensitivity [because total reflectance flux $\Sigma R(\lambda)$ was significantly dimorphic as well]. Sexual dichromatism has never before been reported in an aposematic vertebrate, probably because predators exert stabi-

lizing selection on aposematic signals (37). We know of only one other example of aposematic sexual dichromatism: female-limited mimicry in butterflies (38). This may be explained by higher predation pressure on females in combination with stabilizing sexual selection maintaining the ancestral color pattern in males (39, 40). For *O. pumilio*, our experiments suggest that the brightness dimorphism we observed in Solarte frogs may be the product of sexual selection by female choice.

Despite significant preferences for brighter males in Bastimentos females, we did not find sexual dimorphism in this population. Such mismatch between preferences and traits is not uncommon in sexual selection studies (41–44) and may be due to ecological differences between populations that bias or constrain the evolution of sexual traits. Alternatively, our inability to detect dimorphism in *O. pumilio* from Bastimentos could be related to the extreme color diversity in this population. In particular, although orange and green phenotypes are most abundant there, red and yellowish frogs also occur. Given that hue and brightness are interdependent (see below), this implies that female preferences for brighter males could drive male hue outside the categories considered here. This question could be resolved by a systematic analysis of coloration in a large and unbiased sample of individuals.

Female preferences for greater signal quantity (brighter, louder, larger) can be a direct consequence of increased sensory stimulation (29, 45, 46). In *O. pumilio*, preferences for aposematic signal components are probably influenced by predator selection as well. For instance, empirical work shows that increases in aposematic brightness contrast can enhance predator learning (28), and recent theory suggests that aposematic conspicuousness can function as an honest indicator of toxicity (36). Thus, female choice for conspicuous males may increase offspring fitness in terms of predator deterrence, foraging efficiency and/or alkaloid sequestration. In addition, coloration brightness could indicate physical condition as a result of metabolic tradeoffs.

The natural selection consequences of the brightness dimorphism in Solarte frogs are unknown. As mentioned above, females may be at a disadvantage if brighter signals provide better protection from predators. However, this disadvantage may be absent if the visual conspicuousness of both males and females is already at such a high level that additional increments in brightness do not enhance predator learning. In the latter case, natural and sexual selection may act synergistically, driving signal evolution in the same direction but at different selection strengths. The bright-orange frogs from Solarte would be likely candidates for such a mechanism, because they have the highest estimated conspicuousness (Fig. 2). On the other hand, directional sexual selection may drive aposematic signals beyond the range of phenotypes that are recognized and avoided by predators, generating an evolutionary conflict between divergence and convergence (see also ref. 47). Investigations are underway to evaluate how predators respond to color and brightness variation within and between *O. pumilio* populations. Previous studies of predator responses to aposematic color variation in other poison frogs have yielded varied results, ranging from demonstrating relatively little (48) to substantial (49) costs to signal divergence.

We also found one instance of sexual dimorphism in estimated color contrast: At Almirante, females had greater ventral color contrast than males (Fig. S3). This difference barely exceeded the jnd threshold ($\Delta S = 1.05$) and may not be perceptible to the frogs. However, together with the substantial brightness dimorphism at Solarte, these patterns do suggest that sex-specific selection, through mate choice or other processes, cannot be ignored in studies of aposematic evolution (see also refs. 39 and 47).

Although our study focuses on sexual selection on aposematic brightness, it is important to note that selection on brightness

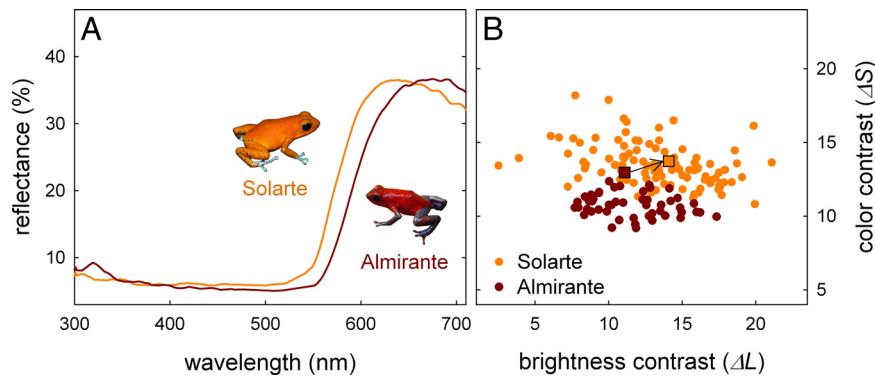


Fig. 3. Dorsal coloration at Almirante and Solarte illustrating the relation between hue and brightness. (A) Dorsal reflectance spectra of two individuals, one from each population, that are matched for baseline and peak reflectance. (B) Color and brightness contrast for an *O. pumilio* viewer under natural light conditions. Circles represent all measured individuals in the two populations, enlarged squares represent the frogs depicted in A. Despite nearly identical baseline and peak reflectance, the orange frog from Solarte has a 10.4% higher total brightness than the red frog from Almirante [$\Sigma R(\lambda)$ Almirante frog = 5,604; Solarte frog = 6,186], resulting in a 27% increase in brightness contrast (ΔL Almirante frog = 11.08; Solarte frog = 14.08).

may contribute to evolutionary changes in hue (color) as well. The phenotypic variation across the *O. pumilio* populations considered here is associated with changes in both brightness and color contrast. In general, creating a brighter visual signal can be achieved via two, nonmutually exclusive design principles: (i) increasing the peak reflectance, i.e., reflecting more light at a given wavelength, or (ii) increasing the spectral width of reflectance, i.e., reflecting light over a broader wavelength range. The increased brightness of Solarte and Bastimentos frogs exemplifies both principles: greater peak reflectances and shorter cut-on wavelengths (wavelength at which reflectance begins to exceed baseline), as compared with the presumably ancestral mainland morph (Almirante). As illustrated in Fig. 3, for example, the orange frogs from Solarte can gain a 27% increase in brightness contrast over red frogs from Almirante, solely as a result of a shorter cut-on wavelength.

The implication of this interdependence between brightness and hue is that directional selection on one coloration trait may cause a concomitant change in the other: selection for increased brightness may cause changes in hue—and vice versa. If selection (sexual or natural) favors brighter signals on the island populations of the Bocas Del Toro archipelago, then one possible evolutionary outcome is a change in hue—from the relatively dark red on the mainland, to the brighter colors observed on the islands (orange, yellow, bright blue, and green). Future studies may explore female preferences for hue variations along a brightness gradient to test the hypothesis that sexual selection for brightness has contributed to the origin of the extreme color polymorphism of *Oophaga pumilio*.

Conclusion

Comparative studies of sexual selection within populations can illuminate the processes that drive population divergence (50–52). Directional sexual selection on male coloration brightness in *O. pumilio* may facilitate sexual dimorphism within populations, which may provide the raw material for interpopulation divergence. Moreover, selection for brightness may cause changes in hue, and populations can show different signatures of this process due to differences in ecological conditions (2) and geographic history (13) and through interaction with genetic drift and founder effects (31, 32). Further work is required to establish whether brightness provides a signal of male quality, how predators respond to signal divergence, and to what extent aposematic brightness and color contrast evolve in concert. Our

findings emphasize that sexual and natural selection must be jointly considered in the study of aposematic evolution.

Experimental Procedures

Animals. Frogs were captured in the field and kept at the Bocas del Toro Field Station of the Smithsonian Tropical Research Institute, Panama. Because *O. pumilio* does not show amplexus, we increased the probability that females were receptive by collecting them in the vicinity of territorial males in the field and selecting females that were relatively heavy. Such females are unlikely to be taking care of a previous clutch. Frogs were housed in individual outdoor terraria and fed a diet of termites, ants, and fruit flies. Fresh water was provided daily. Frogs were measured (snout–vent length, to the nearest 0.1 mm) and weighed (to the nearest 0.01 g) within 2 days of capture.

We used frogs from four populations (Fig. 1A): Isla Solarte (Cayo Nancy), Aguacate Peninsula, the polymorphic Isla Bastimentos, and Almirante [representing the mainland morph, presumably the ancestral phenotype that occurs throughout most of the species range from Nicaragua to Panama (9, 16, 53, 54)].

In October–December 2006, we collected frogs from Solarte ($n = 63$ individuals) and Bastimentos ($n = 62$) for reflectance spectrophotometry. In June–July 2007, we collected frogs from Solarte ($n = 48$), Bastimentos ($n = 70$), Aguacate ($n = 55$, and Almirante ($n = 51$) for reflectance spectrophotometry and mate-choice experiments.

Mate-Choice Experimental Treatments and Setup. Female preferences for brighter males were evaluated in a series of two-way choice experiments. Females were presented with two stimulus males originating from the females' native population. The setup was similar to previous experiments (24) and consisted of three boxes of UV-transparent clear acrylic: one female chamber ($40 \times 20 \times 20$ cm) and two male chambers (half circles with a radius of 10 cm; 20 cm high). The visual background of each male chamber was black (both floor and back wall), to minimize differences in color contrast between stimuli. A visual barrier blocked the males' view of each other throughout the experiment.

Experiments were carried out in an illumination-controlled room, enabling us to manipulate female perception of male brightness by using two different illumination conditions for each male. Both males were illuminated with light that mimics conditions on the forest floor, using three 75-W UV lights and one 25-W halogen light, filtered by two green-blue filters (Lee 728 + CyanGel 4315, ref. 24). For the first 15 min of observations, illumination of one male was reduced by using neutral density filters (one Lee 298 and two GamColor 1514). For the next 15 min, these filters were moved to the other male's chamber, thus reversing the brightness difference between the males. We reduced male brightness by 66%, which is well within the natural range of male brightness in all tested populations: The average standard deviation of male brightness across the four studied populations was (mean $SD \pm SE$) $29.9 \pm 2.9\%$ (for frog radiance spectra in natural and experimental conditions see Fig. S1). Because Aguacate frogs are quite dark dorsally (Figs. 1 and 2), we added an LED lamp to the bright treatment in the Aguacate trials resulting in a larger difference between

the bright and dark illumination (78%). Control experiments used the same light treatments but with empty male chambers to control for a possible female tendency to approach darker or brighter areas.

To reduce the potentially confounding effect of male calling behavior on female preferences (see also ref. 24), we used soundproofing foam around the experimental chambers and broadcast male calls (recorded in the population being tested) from a speaker behind the male compartments to override the sound produced by the stimulus males (for analysis of acoustic effects see *SI Text*).

Mate-Choice Experimental Procedure. Male pairs were of fixed composition (i.e., pairs consisted of the same two individuals for each tested female). Paired males were matched for size, weight, and dorsal reflectance spectra (all differences between males were within one standard deviation of the population mean). For the polymorphic Bastimentos population, females were tested with males of similar color contrast (ΔS , see color analysis below; the mean \pm SE difference in ΔS between tested female and presented males was 0.90 ± 0.19 , i.e., within one standard deviation of the population mean (1.33) and below the *jnd* threshold).

Females were tested for both experimental and control trials on separate days with no female tested more than once per day; male pairs were used for a maximum of four experiments per day. Experimental procedures followed ref. 24. Frogs acclimated to the experimental chambers for at least 60 min (females) or 10 min (males) without visual contact. After acclimation, visual barriers were removed, and the female was placed under a glass in the middle of her chamber to allow observation of both males for 2 min. The glass was then lifted, and observation started when the female approached one of the males to a distance of less than two body lengths (i.e., “interaction zone”; within ≈ 4 cm). Once females had entered this zone, we recorded for 15 min the time that the female spent in the interaction zone of each male (“interaction time”) and counted the number of times that females approached each male chamber. Field observations suggest that physical proximity and female approach behavior are good correlates of mating probability: Successful courtship involves prolonged behavioral interactions that consist of repeated approaches and turning away of the female, followed by repeated attempts of the male to lead her to an egg deposition site (ref. 55 and personal observation). After 15 min, the experimental procedure was repeated immediately with the position of the filters reversed: Visual barriers were inserted again, filters were switched, and the female was confined under the glass again for 5 sec, after which the barriers were removed and the observation started again. If females did not enter the interaction zone within 15 min, they were returned to the housing terraria, and the data were not used.

Across all populations tested, 43% of the females entered the interaction zone within 15 min of observation (similar to previous studies, refs. 6, 23, and 24). The resulting sample sizes are: Solarte (experiments: 24 females and 5 male pairs; controls: 21 females); Bastimentos (experiments: 20 females and 7 male pairs; controls: 19 females); Aguacate (experiments: 20 females and 5 male pairs; controls: 17 females); Almirante (experiments: 17 females and 6 male pairs; controls: 15 females). Most females were tested in both experiment and control treatments (Solarte, 19 females; Bastimentos, 17; Aguacate, 17; Almirante, 11).

Analysis of Frog Coloration: Reflectance Measures, Brightness and Hue Estimates. We measured spectral reflectances, $R(\lambda)$, using a StellarNet EPP200C UV-VIS spectrometer, SL-4 Xenon lamp, and a R400–7 reflectance probe; and used a 2-m 600- μ m diameter fiber optic and cosine collector attachment for habitat irradiance measures for target and background radiance estimates (see below). Spectralon white standard measurements were taken between individuals to account for lamp drift. Dorsal reflectance spectra were obtained by averaging measurements of the head, dorsum, and lower dorsum (two measurements per region). Ventral reflectance spectra were obtained by averaging two measurements each of the upper throat and belly. Individuals with incomplete measurement series were excluded from analyses.

To measure “brightness” differences between populations and between males and females, we evaluated (i) the difference in total reflectance flux ($\sum_{\lambda=300}^{700\text{nm}} R(\lambda)$), and (ii) the estimated difference in brightness contrast (ΔL) of frog coloration when viewed against a natural background by an *O. pumilio* viewer. For the latter, we incorporated the absorbance spectra of three photoreceptor cone classes of *O. pumilio* (56; kindly provided by T. Cronin, University of Maryland, Baltimore, County, Baltimore, MD), irradiance measurements collected in an *O. pumilio* habitat, and reflectance measurements of a common *O. pumilio* background (green *Heliconia* sp. leaf) into a *O. pumilio*-specific visual model that

evaluates conspicuousness as an estimate of luminance (\approx brightness) and spectral (\approx color) differences with the background.

Inputs to the *O. pumilio*-specific visual model include target and background reflectances [$R_t(\lambda)$: *O. pumilio* dorsal and ventral regions; $R_b(\lambda)$: *Heliconia* sp. leaf] along with a noontime downwelling irradiance [$I_D(\lambda)$] measurement collected within a *Heliconia* thicket 2 m above ground under partly-cloudy skies (see Fig. S2 for background and ambient light spectra). These measurements were used to estimate target and background radiance [$\text{Rad}(\lambda) = (R(\lambda) \times I_D(\lambda))/2\theta$], which represents the spectrum of light arriving at the eye surface from a solid viewing angle (here, 180°). Photoreceptor quantum catch, $Q_c(\lambda)$, for target or background radiance was evaluated as $Q_c(\lambda) = \int_{300}^{700} \text{Rad}(\lambda) \times A_c(\lambda) dx$, integrated over 1-nm intervals from 300 to 700 nm, where $A_c(\lambda)$ represents the absorbance spectrum for photoreceptor class *c* [short-wavelength sensitive (SWS), middle-wavelength sensitive (MWS), or long-wavelength sensitive (LWS)].

The *O. pumilio*-specific visual model (56) assumes that discrimination in both the luminance and chromatic (or color) channels is limited by receptor noise and estimates frog photoreceptor noise from behavioral data in other vertebrates (assuming a Weber fraction of 0.05 at threshold for the LWS cones). The model calculates the ability of frogs to discriminate target from background in terms of signal-to-noise ratios (ΔL in the luminance or brightness channel, and ΔS in the chromatic or color channel), which represent estimates of *jnd* where values < 1 are considered below the threshold of discrimination. In brief, the model assumes that photoreceptors adjust to the ambient light environment via a von Kries transformation [$q_c = Q_c(\lambda)/I(\lambda)$] and then evaluates the contrast between target and background spectra for these adapted photoreceptor responses: $\Delta f_c = \ln[q_c(\text{target}) - q_c(\text{background})]$. Brightness contrast, ΔL , or the ability to discriminate target from background in the luminance channel, is assumed to be governed by the LWS cone class only as found in bird systems (57). Hence, signal-to-noise estimates in the luminance channel were evaluated with $\Delta L = |\Delta f_{LWS}/\omega_{LWS}|$, where the noise value, ω , is based on its individual Weber fraction, v , and photoreceptor proportion, n , for that cone class: ($\omega_c = v/n$). Statistical analyses were then applied (see below) to evaluate differences in brightness contrast estimates between groups, reporting only differences above the perceptual threshold (*jnd* > 1).

We also calculated color contrast estimates (ΔS) from an *O. pumilio*-specific perspective (56), as follows (*S*, *M*, and *L* represent the SWS, MWS, and LWS cone classes, respectively):

$$\Delta S = \sqrt{\frac{\omega_S^2(\Delta f_L - \Delta f_M)^2 + \omega_M^2(\Delta f_L - \Delta f_S)^2 + \omega_L^2(\Delta f_S - \Delta f_M)^2}{(\omega_S\omega_M)^2 + (\omega_S\omega_L)^2 + (\omega_M\omega_L)^2}}$$

Data Analysis. All statistical tests were carried out in R software (14). Female preferences were analyzed by using generalized linear mixed models (lme4 package) with binomial error distribution and logit link function adjusted for dispersion. All models included factors for individual females, nested within population, as random effects. Significance of fixed effects was determined by using χ^2 tests comparing alternative models. We tested whether females preferred the more brightly illuminated male, using interaction time and approach frequencies as dependent variables. Models comparing experiments and controls included total interaction time and total approach frequency as additional fixed effects, to control for differences in female responsiveness between these two treatments.

Both estimates of brightness, total reflectance flux [$\sum R(\lambda)$] and brightness contrast estimates (ΔL , along with color contrast estimates, ΔS) of male and female reflectance spectra were compared by using *t* tests (Aguacate and Almirante) and ANOVAs including “sampling year” as a covariate (Bastimentos and Solarte). Brightness differences between populations were analyzed in ANOVAs and subsequent pairwise comparison in Tukey’s HSD tests, using the data from 2007 only.

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1. Ruxton GD, Sherratt TN, Speed M (2005) *Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals and Mimicry* (Oxford Univ Press, Oxford), p 264.
2. Mappes J, Marples N, Endler JA (2005) The complex business of survival by aposematism. *Trends Ecol Evol* 20:598–603.
3. Mallet J, Joron M (1999) Evolution of diversity in warning color and mimicry: Polymorphisms, shifting balance, and speciation. *Annu Rev Ecol Syst* 30:201–233.
4. Ratcliffe JM, Nydam ML (2008) Multimodal warning signals for a multiple predator world. *Nature* 455:96–U59.
5. Jiggins CD, Naisbit RE, Coe RL, Mallet J (2001) Reproductive isolation caused by colour pattern mimicry. *Nature* 411:302–305.
6. Summers K, Symula R, Clough M, Cronin T (1999) Visual mate choice in poison frogs. *Proc R Soc London Ser B* 266:2141–2145.
7. Lande R, Arnold SJ (1985) Evolution of mating preference and sexual dimorphism. *J Theor Biol* 117:651–664.
8. Grant T, et al. (2006) Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia:Athesphatanura:Dendrobatidae). *Bull Am Mus Nat Hist* 6:262.
9. Savage JM (1968) The Dendrobatid frogs of Central America. *Copeia* 4:745–776.
10. Daly JW, Myers CW (1967) Toxicity of Panamanian poison frogs (*Dendrobates*)—Some biological and chemical aspects. *Science* 156:970–973.
11. Myers CW, Daly JW (1983) Poison dart frogs. *Sci Am* 248(2):120–133.
12. Summers K, Cronin TW, Kennedy T (2003) Variation in spectral reflectance among populations of *Dendrobates pumilio*, the strawberry poison frog, in the Bocas del Toro Archipelago, Panama. *J Biogeogr* 30:35–53.
13. Anderson RP, Handley CO (2002) Dwarfism in insular sloths: Biogeography, selection, and evolutionary rate. *Evolution (Lawrence, Kans)* 56:1045–1058.
14. R Development Core Team (2008) R: A language and environment for statistical computing. (R Foundation for Statistical Computing, Vienna, Austria).
15. Summers K, Bermingham E, Weigt L, McCafferty S (1997) Phenotypic and genetic divergence in three species of dart-poison frogs with contrasting parental behavior. *J Hered* 88:8–13.
16. Wang JJ, Shaffer HB (2008) Rapid color evolution in an aposematic species: A phylogenetic analysis of color variation in the strikingly polymorphic strawberry poison-dart frog. *Evolution (Lawrence, Kans)* 62:2742–2759.
17. Lande R (1980) Genetic-variation and phenotypic evolution during allopatric speciation. *Am Nat* 116:463–479.
18. Mayr E (1963) *Animal Species and Evolution* (Harvard Univ Press, Cambridge, MA).
19. Whittaker RJ (1998) *Island Biogeography: Ecology, Evolution and Conservation*. (Oxford Univ Press, Oxford).
20. Saporito RA, et al. (2007) Spatial and temporal patterns of alkaloid variation in the poison frog *Oophaga pumilio* in Costa Rica and Panama over 30 years. *Toxicon* 50:757–778.
21. Saporito RA, Zuercher R, Roberts M, Gerow KG, Donnelly MA (2007) Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia* 1006–1011.
22. Fritz G, Rand AS, Depamphilis CW (1981) The aposematically colored frog, *Dendrobates pumilio*, is distasteful to the large, predatory ant, *Paraponera clavata*. *Biotropica* 13:158–159.
23. Reynolds RG, Fitzpatrick BM (2007) Assortative mating in poison-dart frogs based on an ecologically important trait. *Evolution (Lawrence, Kans)* 61:2253–2259.
24. Maan ME, Cummings ME (2008) Female preferences for aposematic signal components in a polymorphic poison frog. *Evolution (Lawrence, Kans)* 62:2334–2345.
25. Pröhl H, Hodl W (1999) Parental investment, potential reproductive rates, and mating system in the strawberry dart-poison frog, *Dendrobates pumilio*. *Behav Ecol Sociobiol* 46:215–220.
26. Trivers RL (1972) Parental investment and sexual selection. *Sexual Selection and the Descent of Man*, ed Campbell B (Aldine-Atherton, Chicago), pp 136–179.
27. Lande R (1981) Models of speciation by sexual selection on polygenic traits. *Proc Natl Acad Sci USA* 78:3721–3725.
28. Prudic KL, Skemp AK, Papaj DR (2007) Aposematic coloration, luminance contrast, and the benefits of conspicuousness. *Behav Ecol* 18:41–46.
29. Ryan MJ, Keddy-Hector A (1992) Directional patterns of female mate choice and the role of sensory biases. *Am Nat* 139:54–535.
30. Marchetti K (1993) Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* 362:149–152.
31. Garcia-Ramos G, Kirkpatrick M (1997) Genetic models of adaptation and gene flow in peripheral populations. *Evolution (Lawrence, Kans)* 51:21–28.
32. Gavrillets S (2003) Perspective: Models of speciation: What have we learned in 40 years? *Evolution (Lawrence, Kans)* 57:2197–2215.
33. Pröhl H, Hageman S, Karsch J, Hoebel G (2007) Geographic variation in male sexual signals in stawberry poison frogs (*Dendrobates pumilio*). *Ethology* 113:825–837.
34. Endler JA, Mappes J (2004) Predator mixes and the conspicuousness of aposematic signals. *Am Nat* 163:532–547.
35. Labeyrie E, Blanckenhorn WU, Rahier M (2003) Mate choice and toxicity in two species of leaf beetles with different types of chemical defense. *J Chem Ecol* 29:1665–1680.
36. Blount JD, Speed MP, Ruxton GD, Stephens PA (2009) Warning displays may function as honest signals of toxicity. *Proc R Soc London Ser B* 276:871–877.
37. Müller F (1879) *Ituna* and *Thyridia*: A remarkable case of mimicry in butterflies. *Proc Entomol Soc London* 1879:xx–xxx.
38. Wallace AR (1865) On the phenomena of variation and geographical distribution as illustrated by the Papilionidae of the Malayan region. *Trans Linnæan Soc* 25:1–71.
39. Kunte K (2008) Mimetic butterflies support Wallace's model of sexual dimorphism. *Proc R Soc London Ser B* 275:1617–1624.
40. Ohsaki N (1995) Preferential predation of female butterflies and the evolution of Batesian mimicry. *Nature* 378:173–175.
41. Hill GE, McGraw KJ (2004) Correlated changes in male plumage coloration and female mate choice in cardueline finches. *Anim Behav* 67:27–35.
42. Ryan MJ, Rand AS (1993) Sexual selection and signal evolution—The ghost of biases past. *Phil Trans R Soc London Ser B* 340:187–195.
43. Houde AE, Hankes MA (1997) Evolutionary mismatch of mating preferences and male colour patterns in guppies. *Anim Behav* 53:343–351.
44. Simmons LW, Zuk M, Rotenberry JT (2001) Geographic variation in female preference functions and male songs of the field cricket *Teleogryllus oceanicus*. *Evolution (Lawrence, Kans)* 55:1386–1394.
45. Endler JA (1992) Signals, signal conditions, and the direction of evolution. *Am Nat* 139:5125–5153.
46. Ryan MJ, Fox JH, Wilczynski W, Rand AS (1990) Sexual selection for sensory exploitation in the frog *Phrylosalaemus pustulosus*. *Nature* 343:66–67.
47. Estrada C, Jiggins CD (2008) Interspecific sexual attraction because of convergence in warning coloration: Is there a conflict between natural and sexual selection in mimetic species? *J Evol Biol* 21:749–760.
48. Darst CR, Cummings ME (2006) Predator learning favours mimicry of a less-toxic model in poison frogs. *Nature* 440:208–211.
49. Noonan BP, Comeault AA (2009) The role of predator selection on polymorphic aposematic poison frogs. *Biol Lett* 5:51–54.
50. West-Eberhard MJ (1983) Sexual selection, social competition, and speciation. *Q Rev Biol* 58:155–183.
51. Ritchie MG (2007) Sexual selection and speciation. *Annu Rev Ecol Syst* 38:79–102.
52. Gray SM, McKinnon JS (2007) Linking color polymorphism maintenance and speciation. *Trends Ecol Evol* 22:71–79.
53. Hageman S, Pröhl H (2007) Mitochondrial paralogy in a polymorphic poison frog species (*Dendrobatidae*; *D. pumilio*). *Mol Phylogenet Evol* 45:740–747.
54. Walls JG (1994) *Poison Frogs of the Family Dendrobatidae: Jewels of the Rainforest* (TFH Publications, Neptune City, NJ), p 288.
55. Limerick S (1980) Courtship behavior and oviposition of the poison-arrow frog *Dendrobates pumilio*. *Herpetologica* 36:69–71.
56. Siddiqi A, Cronin TW, Loew ER, 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.
57. Maier EJ, Bowmaker JK (1993) Color vision in the Passeriform bird, *Leiothrix Lutea*—Correlation of visual pigment absorbency and oil droplet transmission with spectral sensitivity. *J Comp Physiol A* 172:295–301.