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# Warning Signal Brightness Variation: Sexual Selection May Work under the Radar of Natural Selection in Populations of a Polytypic Poison Frog

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**ABSTRACT:** Though theory predicts consistency of warning signals in aposematic species to facilitate predator learning, variation in these signals often occurs in nature. The strawberry poison frog *Dendrobates pumilio* is an exceptionally polytypic (populations are phenotypically distinct) aposematic frog exhibiting variation in warning color and brightness. In the Solarte population, males and females both respond differentially to male brightness variation. Here, we demonstrate through spectrophotometry and visual modeling that aposematic brightness variation within this population is likely visible to two putative predators (crabs, snakes) and conspecifics but not to the presumed major predator (birds). This study thus suggests that signal brightness within *D. pumilio* populations can be shaped by sexual selection, with limited opportunity for natural selection to influence this trait due to predator sensory constraints. Because signal brightness changes can ultimately lead to changes in hue, our findings at the within-population level can provide insights into understanding this polytypism at across-population scales.

**Keywords:** visual model, aposematism, brightness, conspicuousness, predation, variation.

## Introduction

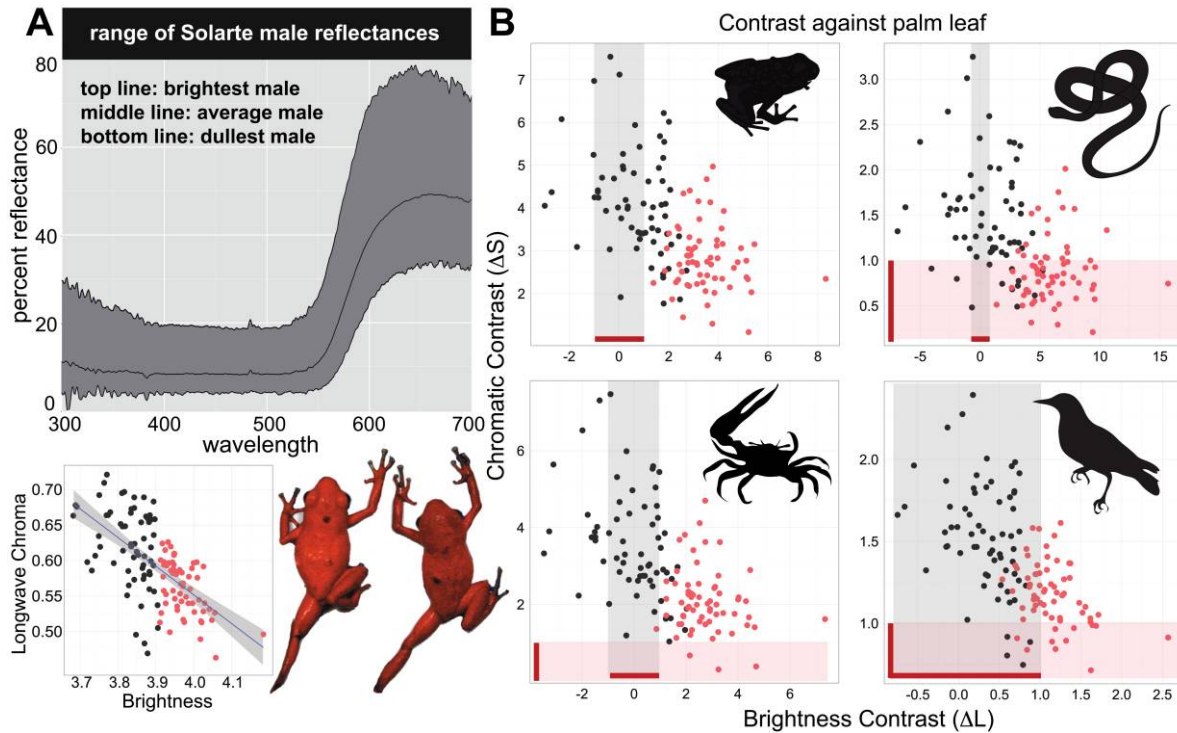
Conspicuous traits typically evolve through an antagonistic interplay between sexual selection and predation (Darwin 1887; Endler 1992). Yet in aposematic species, which use warning signals to deter predators (Ruxton et al. 2004), the interaction of these forces on conspicuousness is not as clear. Considerable intra- and interpopulation variation in warning coloration has been observed across a diversity of taxa (e.g., Bezzerides et al. 2007; Speed et al. 2010; reviewed in Ruxton et al. 2004). Recent work suggests that aposematic traits can be co-opted as sexual signals (Jiggins et al. 2001) and evolve via sexual selection (Maan and

Cummings 2009). Since these traits often advertise to multiple predators featuring diverse sensory systems (Endler and Mappes 2004), variation in aposematic signals driven by sexual selection is predicted to be opposed by predators (Müller 1879). However, the interaction between these two selective forces on such signals is relatively unknown, and the perceptibility of aposematic signal variation to predators is underinvestigated (Stevens 2007; Lindstedt et al. 2011; Maan and Cummings 2012).

The aposematic strawberry poison frog *Dendrobates (Oophaga) pumilio* exhibits extreme warning color variation in the Bocas del Toro archipelago of Panama, with ~15 distinct phenotypes represented across island and mainland populations (polytypism; Daly and Myers 1967; Siddiqi et al. 2004). Assortative mating of color morphs within populations and directional sexual selection on male coloration and brightness across populations have both been implicated in the evolution of this variation (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Rudh et al. 2007; Maan and Cummings 2008, 2009). One of the best-studied populations (Solarte) contains orange-red frogs representing one of the brightest morphs in the archipelago (fig. 1A; second brightest of 10 populations; Maan and Cummings 2012). While both Solarte males and females are exceptionally bright, this population is also sexually dimorphic in terms of aposematic brightness, and both sexes exhibit differential behavioral responses to male brightness variation in the population (Maan and Cummings 2009; Crothers et al. 2011). Taken together, these results suggest that this phenotypic feature is under the influence of sexual selection. Here, we examine whether this aposematic brightness variation, likely generated by sexual selection, is detectable to the putative predators of *D. pumilio*.

Purifying selection against rare aposematic phenotypes has been observed in polymorphic insects (Kapan 2001;

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**Figure 1:** Perceptually unbiased (A) and taxon-specific estimates (B) of color and brightness. A, Variation in reflectance across different wavelengths of Solarte males. Dark region of line plot represents the observed range of Solarte male reflectances. The scatter plot represents the relationship between two perceptually unbiased measures: brightness (log of the total reflectance flux) and long-wave chroma, the proportion of long-wave (600–700 nm) reflectance relative to the total. The accompanying photographs depict two male frogs representing some of the phenotypic variation observed in the population. B, Brightness contrast plotted against chromatic contrast against a dry palm leaf background. For all scatter plots, dots are color coded according to perceptually unbiased reflectance flux brightness classes (red, brighter than average; black, duller than average). Shaded areas of the plots indicate regions of perceptual contrast space in which individuals are likely to be indiscriminable from the background in that particular channel (gray, brightness contrast; red, chromatic contrast; values are below the signal detection threshold,  $<1.0$ ).

Borer et al. 2010) and other polytypic poison frog species (Noonan and Comeault 2009; Chouteau and Angers 2011; Comeault and Noonan 2011), suggesting that such forces could also be at play in *D. pumilio*. Natural selection by predators may therefore interact with sexual selection in this species, effectively limiting any deviations from the average aposematic phenotype within a given population. Though the main predators of *D. pumilio* in the Bocas del Toro region are still unknown, clay frog predation experiments have implicated birds as the major predator of several poison frog species (Noonan and Comeault 2009; Chouteau and Angers 2011; Comeault and Noonan 2011) as well as *D. pumilio* in other parts of the species' geographic range (Saporito et al. 2007; Hegna et al. 2011). While birds are capable of detecting differences between the different *D. pumilio* morphs (Maan and Cummings 2012; M. E. Cummings, unpublished data), no studies have yet investigated whether within-population variation in brightness is also detectable to predators. Such investi-

gations can elucidate the relative roles played by natural selection and sexual selection in the phenotypic divergence of this species. Here, we use taxon-specific visual modeling analyses of the conspecific and three predator visual systems, using spectrophotometric measurements of Solarte *D. pumilio* males and six perch backgrounds to investigate the perceptibility of intrapopulation aposematic brightness variation. This study aims to (1) determine whether potential predators are likely to perceive brightness variation in male *D. pumilio* of the Solarte population and (2) determine whether variation in perch site backgrounds affects the discriminability of this variation in male conspicuousness.

## Methods

### Collection and Spectral Measurements

Solarte *Dendrobates pumilio* males ( $N = 128$ ) were captured in July–August 2009 and measured in a temperature-

controlled room at the Smithsonian Tropical Research Institute in Bocas del Toro, Panama, within 24 h of capture. Spectral reflectance measurements were taken at the head and dorsum (two measurements per region) using an EPP200C UV-VIS spectrometer, SL-4 Xenon lamp, and R400-7 reflectance probe at a 3-mm distance for frogs and a 2-mm distance for substrates (StellarNet, Tampa, FL). Spectralon white standard measurements were taken between frogs to account for lamp drift.

#### *Analysis of Brightness and Coloration*

*Perceptually Unbiased Measures.* Dorsal measurements ( $N = 4$ ) were averaged for each frog. We first calculated inherent measures of brightness (log of total reflectance flux),

$$\log \left( \sum_{i=300 \text{ nm}}^{700 \text{ nm}} R(\lambda)_i \right),$$

and color (long-wave chroma) to evaluate brightness and coloration using spectrophotometry. Long-wave chroma assesses the proportion of the reflectance flux in the long-wave band (600–700 nm):

$$\frac{\sum_{i=600 \text{ nm}}^{700 \text{ nm}} R(\lambda)_i}{\sum_{i=300 \text{ nm}}^{700 \text{ nm}} R(\lambda)_i}.$$

Last, we calculated the inherent radiance contrast of a frog against its background,

$$\frac{Q_{\text{frog}} - Q_{\text{background}}}{Q_{\text{frog}} + Q_{\text{background}}},$$

where  $Q = \int_{\lambda=300}^{700} I(\lambda)R(\lambda)d\lambda$ ,  $I(\lambda)$  is the habitat irradiance, and  $R(\lambda)$  is the reflectance.

*Taxon-Specific Measures.* To assess the perceptual consequences of brightness variation in a taxon-specific manner, we estimated the contrast of frogs against different backgrounds using receptor-based visual models. Visual models provide an approximation of an animal's perception of visual information, have predicted behavioral sensitivities in a variety of taxa (Vorobyev and Osorio 1998; Vorobyev et al. 2001; Goldsmith and Butler 2003), and can provide reliable estimates of predation risk (Stuart-Fox et al. 2003; Husak et al. 2006; Stobbe and Schaefer 2008).

Frog predators come from widely different taxa (e.g., Silverstone 1975; Myers and Daly 1976). Accounts of attacks on *D. pumilio*, though rare, implicate forest crabs, birds, spiders, and snakes as potential predators (M. E. Cummings, unpublished data). Our predator visual models include previously described passerine, crab, and diurnal snake models (Cummings et al. 2008; Maan and

Cummings 2012) as well as a conspecific visual model (based on Siddiqi et al. 2004; Maan and Cummings 2009, 2012; Crothers et al. 2011). Our visual modeling methods are based on receptor noise-limited models originally developed by Vorobyev and Osorio (1998). Each model involves steps and equations described previously (Cummings et al. 2008; Maan and Cummings 2009, 2012), incorporating frog and background reflectance, ambient light, and receiver visual sensitivities into conspicuousness calculations and assuming that visual detection ability is limited by photoreceptor noise (data available in Dryad: <http://dx.doi.org/10.5061/dryad.p5g5j>). In brief, the models use the following steps:

Photoreceptor quantum catch  $Q_c$  for target (frog) or background (substrate) radiance is calculated as the integrated product of habitat irradiance  $I(\lambda)$ , target or background reflectance  $R(\lambda)$ , and photoreceptor cone absorbance  $A_c(\lambda)$  for each cone class  $c$ :

$$Q_c = \int_{\lambda=300}^{700} I(\lambda)R(\lambda)A_c(\lambda)d\lambda,$$

integrated over 1-nm intervals from 300 to 700 nm. Quantum catch is adjusted for the adapting light environment using von Kries transformations, such that  $q_c = k_c Q_c$  and

$$k_c = \frac{1}{\int_{\lambda=300}^{700} I_b(\lambda)A_c(\lambda)d\lambda},$$

where  $I_b(\lambda)$  is the adapting visual background (=habitat irradiance). Photoreceptor signal is proportional to the logarithm of these adjusted quantum catches such that contrast between target and background is

$$\Delta f_c = \ln \frac{q_c(\text{target})}{q_c(\text{background})},$$

where background was one of six common substrates in male territories. Substrates used in the analyses were the most common daytime signaling backgrounds used by calling males in this population (see also Pröhl and Ostrowski 2011). We quantified perch site substrate use by finding 82 calling males across June–August of 2011. Calling males were located at their perch sites, and the substrate background on which the male was standing was noted. Substrate samples were then collected for subsequent spectral reflectance measurement. The substrates were then organized into six classes that encapsulate the diversity of spectral characteristics of calling backgrounds in this population: moss (13 males), leaf litter (16), green leaves (11), dry fallen palm leaves (24), soil (5), and tree trunks/branches (13) (for spectra, see fig. A1, available online). All visual model analyses were evaluated using a

habitat irradiance representing the median quantum flux measurement collected across 38 Solarte male territories on an overcast day in August 2009.

Target detection is assumed to be subject to photoreceptor noise ( $\omega$ ), a function of the Weber fraction for each cone class ( $\nu$ ), and the relative number of receptor types in the retina ( $\eta$ ), where  $\omega = \nu/\eta$ . Cone proportions and Weber fractions used for these models have been described previously (Maan and Cummings 2009, 2012).

Color and brightness appear to be processed independently in invertebrates and in vertebrates (Fleishman and Persons 2001; Endler and Mielke 2005; Osorio and Vorobyev 2005; Lind and Kelber 2011); thus, we calculated two separate contrast measures: brightness contrast and chromatic contrast. Brightness contrast ( $\Delta L$ ), the ability to discriminate target from background in the luminance channel, is governed by the long-wavelength sensitive (LWS) cone class in many terrestrial organisms (Maier and Bowmaker 1993) and by the double cones in birds (Endler and Mielke 2005). Signal to noise estimates in the luminance channel were therefore evaluated as  $\Delta L = |\Delta f_{LWS}/\omega_{LWS}|$  for frog, snake, and crab models and as  $\Delta L = |\Delta f_{double}/\omega_{double}|$  for the bird, using the double cone spectral absorbance measures of the starling (kindly provided by N. S. Hart; Hart et al. 1998). This is a departure from our laboratory's previous modeling investigations wherein the LWS cones were used for avian brightness contrast estimates (Maan and Cummings 2009, 2012). Chromatic contrast estimates ( $\Delta S$ ) were evaluated according to the type of visual system, using the following equations: for dichromat (crab),

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

for trichromat (snake, frog),

$$\Delta S =$$

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

and for tetrachromat (bird),

$$\Delta S =$$

$$\{[(\omega_U\omega_S)^2(\Delta f_L - \Delta f_M)^2 + (\omega_U\omega_M)^2(\Delta f_L - \Delta f_S)^2 + (\omega_U\omega_L)^2(\Delta f_M - \Delta f_S)^2 + (\omega_S\omega_M)^2(\Delta f_L - \Delta f_U)^2 + (\omega_S\omega_L)^2(\Delta f_M - \Delta f_U)^2 + (\omega_M\omega_L)^2(\Delta f_S - \Delta f_U)^2]$$

$$/[(\omega_U\omega_S\omega_M)^2 + (\omega_U\omega_S\omega_L)^2 + (\omega_U\omega_M\omega_L)^2 + (\omega_S\omega_M\omega_L)^2]\}^{1/2}.$$

Additional estimates of conspicuousness can be found in the appendix, available online. Estimates of <1 are con-

sidered indistinguishable (less than the signal detection threshold).

### Data Analysis

We first assessed signaling backgrounds of calling males of this population using a  $\chi^2$  goodness of fit test, testing the hypothesis that frogs were distributed equally across substrate categories. We then used visual modeling estimates to assess the ability of viewers to distinguish differences between males, using two approaches: (1) comparison of all possible male pairs across the population and (2) comparison of dull versus bright male classes. For the first approach, we created distance matrixes of pairwise Euclidian distances between all males in the data set against each of the six backgrounds. Wilcoxon signed rank tests were then performed to see whether these distributions of Euclidian distances exceeded 1, allowing us to assess whether population-wide variation in brightness was detectable to these different viewers. We then classified males by their inherent brightness into above (bright;  $N = 64$ ) or below (dull;  $N = 64$ ) population mean log total reflectance flux, and we evaluated these categories with each taxon-specific visual model to determine whether the variation in male brightness between these two classes of males was distinguishable to different viewers.  $\Delta S$  and  $\Delta L$  estimates were thus calculated for all males with each of the substrate backgrounds and taxon-specific visual models. The resulting estimates were then compared between the bright and dull male categories, using Wilcoxon rank sum tests to assess whether the male brightness classes differed by more than the signal detection threshold (null  $\mu < 1$ ). All analyses were performed in R (R Development Core Team 2012).  $P$  values were corrected for multiple comparisons using the Bonferroni-Holm correction.

## Results

### Perch Site Substrates

Males were not equally distributed among the substrate categories ( $\chi^2$  test;  $\chi^2 = 14.29$ ,  $df = 5$ ,  $P = .014$ ). Post hoc investigation indicated that males were found significantly more often on a palm background and less often on a soil background than expected by chance (standardized residuals  $> |2|$ ).

### Perceptually Unbiased Measures of Contrast

Brightness and long-wave chroma (~saturation of redness) exhibited a negative relationship (fig. 1A;  $t = -9.158$ ,  $P \ll .001$ ,  $R^2 = 0.3996$ ). As expected, inherent contrast estimates between the bright and dull male categories were

nonoverlapping and differed significantly against all substrate backgrounds (fig. 2; Wilcoxon rank sum tests, all  $P \ll .001$ ).











*Taxon-Specific Measures*

Pairwise Euclidian distance estimates for all possible male pairings, an assessment of the distinguishability of spectral variation between males, exceeded the signal detection threshold for all visual systems but the bird (values in gray in fig. 2; Wilcoxon signed rank tests; all  $P \ll .001$  for crab, snake, conspecific;  $P = 1$  for bird), indicating that much of the variation between males is likely perceptible to these visual systems but not to avian predators. Analysis of the two inherent male brightness classes (bright and dull) revealed that brightness contrast ( $\Delta L$ ) estimates between males of these categories differed by more than the signal detection threshold against all substrate backgrounds for the crab, snake, and conspecific visual models but not for

the bird (Wilcoxon rank sum tests; all  $P \ll .001$  for crab, snake, conspecific;  $P = 1$  for bird; fig. 2). Furthermore, only a portion of the bright class—and none of the dull—was detectable to the avian viewer in the  $\Delta L$  channel against the background on which males were most commonly found (dry palm; figs. 1B, 2, A2 [available online]). None of the chromatic ( $\Delta S$ ) comparisons between the bright and dull classes differed by more than the signal detection threshold for any of the visual models (figs. 1B, 2).

**Discussion**

Our study indicates that (1) the extensive intermale variation in brightness in the *Dendrobates pumilio* Solarte population is likely detectable to conspecifics and some predators (snakes, crabs) but not to the presumed major predator (birds) and (2) these results are robust to the naturally occurring backgrounds on which males are com-

	Inherent Contrast	Bird 		Snake 		Crab 		Frog 	
		$\Delta L$	$\Delta S$	$\Delta L$	$\Delta S$	$\Delta L$	$\Delta S$	$\Delta L$	$\Delta S$
 Leaf	Bright: 0.278 Dull: 0.147	Bright: <u>0.995</u> (48) Dull: <u>0.302</u> (0)	Bright: 1.514(100) Dull: 1.848(100)	Bright: 4.395(98) Dull: <u>-0.303</u> (33)	Bright: <u>0.712</u> (17) Dull: 1.190(67)	Bright: 2.164(94) Dull: <u>-0.118</u> (8)	Bright: 1.821(92) Dull: 3.129(98)	Bright: 2.859(98) Dull: <u>0.461</u> (36)	Bright: 2.756(100) Dull: 4.071(100)
		0.650		3.507		2.441		2.371	
 Moss	Bright: 0.731 Dull: 0.670	Bright: 3.974(100) Dull: 3.281(100)	Bright: 1.787(100) Dull: 1.898(100)	Bright: 22.746(100) Dull: 18.048(100)	Bright: 1.979(100) Dull: 1.596(94)	Bright: 11.984(100) Dull: 9.703(100)	Bright: 2.212(89) Dull: 1.249(63)	Bright: 12.843(100) Dull: 10.445(100)	Bright: 1.105(61) Dull: 2.311(91)
		0.542		3.460		2.196		2.249	
 Tree	Bright: 0.618 Dull: 0.539	Bright: 3.444(100) Dull: 2.751(100)	Bright: 1.176(86) Dull: 1.421(94)	Bright: 20.623(100) Dull: 15.925(100)	Bright: <u>0.959</u> (34) Dull: 1.051(63)	Bright: 10.069(100) Dull: 7.788(100)	Bright: 0.536(25) Dull: 1.772(80)	Bright: 11.188(100) Dull: 8.791(100)	Bright: 2.116(95) Dull: 3.428(100)
		0.600		3.452		2.345		2.368	
 Leaf Litter	Bright: 0.243 Dull: 0.129	Bright: 1.380(95) Dull: <u>0.687</u> (9)	Bright: 1.340(95) Dull: 1.701(97)	Bright: 7.594(100) Dull: 2.896(75)	Bright: 1.240(81) Dull: 1.760(97)	Bright: 3.248(100) Dull: <u>0.966</u> (47)	Bright: 3.001(100) Dull: 4.308(100)	Bright: 4.156(100) Dull: 1.759(70)	Bright: 3.332(100) Dull: 4.647(100)
		0.678		3.512		2.441		2.375	
 Palm	Bright: 0.237 Dull: 0.122	Bright: 1.085(61) Dull: <u>0.392</u> (0)	Bright: 1.176(83) Dull: 1.538(94)	Bright: 5.626(100) Dull: <u>0.927</u> (48)	Bright: <u>0.840</u> (30) Dull: 1.342(81)	Bright: 2.526(98) Dull: <u>0.245</u> (20)	Bright: 1.994(95) Dull: 3.302(100)	Bright: 3.247(100) Dull: <u>0.850</u> (45)	Bright: 2.729(100) Dull: 4.044(100)
		0.666		3.510		2.441		2.373	
 Soil	Bright: 0.786 Dull: 0.737	Bright: 5.213(100) Dull: 4.520(100)	Bright: 1.424(100) Dull: 1.573(100)	Bright: 31.915(100) Dull: 27.217(100)	Bright: 2.227(100) Dull: 2.172(100)	Bright: 15.555(100) Dull: 13.274(100)	Bright: 0.416(20) Dull: 1.503(69)	Bright: 17.094(100) Dull: 14.696(100)	Bright: 2.118(95) Dull: 3.429(100)
		0.558		3.441		2.282		2.367	

**Figure 2:** Median contrast estimates for three putative predator visual systems and conspecifics. Values in table cells are calculated separately for bright (>mean log total reflectance flux) and dull (<mean log total reflectance flux) male classes. Adjacent values in parentheses indicate the percentage of individuals that exceeded the signal detection threshold for each class. Underlined values do not exceed the signal-noise detection threshold (1.0). Shaded cells are those where the values for the two male brightness classes are likely to be indistinguishable (difference <1, as indicated by Wilcoxon rank sum tests). Values along the bottoms of the cells contained in circular gray boxes represent the median Euclidian distance taken from pairwise contrast matrixes for all possible male pairings.

monly found (figs. 1, 2). While these findings are based on theoretical visual modeling, studies comparing receptor noise-limited models with behaviorally measured sensitivities have demonstrated these models' usefulness in birds (e.g., Goldsmith and Butler 2003) and other taxa (Vorobyev et al. 2001). Our frog model results also corroborate behavioral responses of conspecifics to brightness variation in this population (bright vs. dull males; Maan and Cummings 2009; Crothers et al. 2011). Hence, these results likely provide realistic estimates for actual perceptual differences in the wild.

There is mounting evidence that variation in aposematic coloration is common (fig. 1; Bezzerides et al. 2007; Speed et al. 2010) and that both color (reviewed in Ruxton et al. 2004) and brightness (Prudic et al. 2007) are important signal components to which predators attend. In *D. pumilio*, male brightness is a salient cue during male territorial interactions (Crothers et al. 2011) and may be undergoing directional sexual selection in at least some populations of this species (Maan and Cummings 2009). Though sexual selection has evidently impacted the evolution of coloration in *D. pumilio*, the species' conspicuous phenotype also functions as an aposematic signal (Saporito et al. 2007). Phenotypic variation has not been observed in other syntopic poison frog species (Summers et al. 1997). However, the variation in dorsal brightness across *D. pumilio* populations in Bocas del Toro appears both detectable and informative (in terms of toxicity level) to potential predators, particularly birds (Maan and Cummings 2012), suggesting a potential for predators to influence this color variation across the archipelago. Yet interestingly, our study suggests that these same predators (birds) are unlikely to detect the variation in signal brightness within one of the brightest populations.

Birds are considered an important predator of poison frogs (Noonan and Comeault 2009; Chouteau and Angers 2011; Comeault and Noonan 2011), including *D. pumilio* (Saporito et al. 2007; Hegna et al. 2011). Therefore, the information provided by Solarte's brightness variation, though accessible to conspecifics, appears to be indiscriminable to its presumed major predator (figs. 1B, 2, A2). However, despite this sensory constraint, it is possible for avian predators to exert selection on brightness variation in this population indirectly. Brighter Solarte males approach rival males faster than duller males (Crothers et al. 2011). These behavioral correlates of brightness may allow avian predators to exert selective pressure on brightness by differentially preying on more active males.

To understand the mechanisms driving the evolution of warning signals, it is necessary to analyze warning phenotypes not only within the framework of predator perception but also against common signaling backgrounds. We found that Solarte males' brightness variation was al-

ways discriminable to the conspecific, snake, and crab visual systems but never to the bird visual system, against all of the signaling backgrounds that we identified ( $\Delta L$ ; fig. 2). Brightness and chromatic components of a visual scene are often used in different ways. Brightness information is typically used to detect small objects, track movement, and resolve pattern details, while chromatic information is used to identify the spectral features of materials and discriminate large targets (Osorio et al. 1999; Jones and Osorio 2004; Endler and Mielke 2005). It is therefore assumed that achromatic (brightness) vision dominates at large distances, while chromatic information is accessible when an animal is closer to its target (Campenhuisen and Kirschfeld 1998; Osorio et al. 1999; Defrize et al. 2010; though see Schaefer et al. 2006; Stobbe et al. 2009; Lind and Kelber 2011). This implies that at a distance many frogs may be undetectable (especially on palms, their most common signaling background) and that signal variation between males in this population may be particularly indistinguishable to birds (figs. 2, A2).

The mechanisms maintaining Solarte brightness variation remain unclear, yet the current data favor sexual selection as a major contributor. Research into the physiological correlates of dorsal brightness in this population suggests that brightness may be a condition-dependent trait. Solarte male brightness does not correlate with body mass, length, or a traditional metric of condition (length-mass residuals); however, it does correlate with body temperature, an important trait in ectotherms (Crothers et al. 2011). Furthermore, the coloration and brightness of red/orange *D. pumilio* morphs can change in captivity over long time periods, indicating that at least some component of coloration may be dietarily based (L. R. Crothers, unpublished data; Summers et al. 2003; J. Yeager and C. Richards-Zawacki, unpublished data). If dorsal brightness is condition dependent in this population, then we should not be surprised to see that both males and females exhibit variation in this trait (see Maan and Cummings 2009). Furthermore, the sexual dimorphism we observe in this trait is also expected in this species, where males are under greater sexual selection pressures than females through the combined effect of females being choosier (because of greater parental investment) and males experiencing strong intrasexual selection (Pröhl and Hödl 1999).

Finally, the results of our study, though limited to a population exhibiting a single color pattern, provide insights into the selective mechanisms of the species' color diversification. Though the relative roles of male- and female-mediated sexual selection are under active investigation, if sexual selection on *D. pumilio*'s aposematic phenotype continues in a unidirectional fashion to enhance male brightness, chromatic aspects of the signal are also likely to be impacted. Maan and Cummings (2009)

hypothesized that interdependence between brightness and hue in the aposematic coloration of *D. pumilio*—coupled with sexual selection on small, isolated populations—may account for the change in hue observed across island populations of Bocas del Toro. Our measurements here show that changes in inherent brightness result in a concomitant change in chromatic properties within a single population. We found a negative relationship between long-wave chroma (~redness) and brightness (fig. 1A) in the Solarte population and a complementary trade-off between chromatic contrast and brightness contrast for the taxon-specific viewers (fig. 1B). This coupling of chroma and brightness suggests that the previously documented sexual selection on brightness could play a key role in the diversification of hues in isolated populations of this species.

Our study suggests that natural selection and sexual selection may predominantly be impacting aposematic coloration at different scales in *D. pumilio*. The inability of the species' major predator (birds) to detect the extensive brightness variation found within this population hints at a permissiveness of predators toward ongoing signal evolution promoted by conspecifics. Thus, variation in signal brightness may be shaped by sexual selection working below the radar of natural selection at the within-population (micro) scale. However, this trait is evidently influenced by natural selection at a larger (macro) scale across populations, where there is a strong relationship between morph toxicity and dorsal brightness across *D. pumilio* morphs, and this relationship appears to be more easily discriminable to birds than other viewers (Maan and Cummings 2012). We have also shown that sexually selected changes on signal brightness within a single population can lead to concomitant changes in another signal attribute (hue/color). This phenomenon may lead to macro-level effects on the aposematic signal, with larger incremental changes in aposematic brightness leading to hue changes that are observable to predators. Hence, signal brightness within *D. pumilio* populations may be shaped by sexual selection, with limited opportunity for natural selection to influence this trait because of predator sensory constraints. Taken together, our study suggests that sexual selection may generate the direction and microtuning of aposematic trait evolution in some populations of this species while natural selection acts as a purifying agent at coarser scales.

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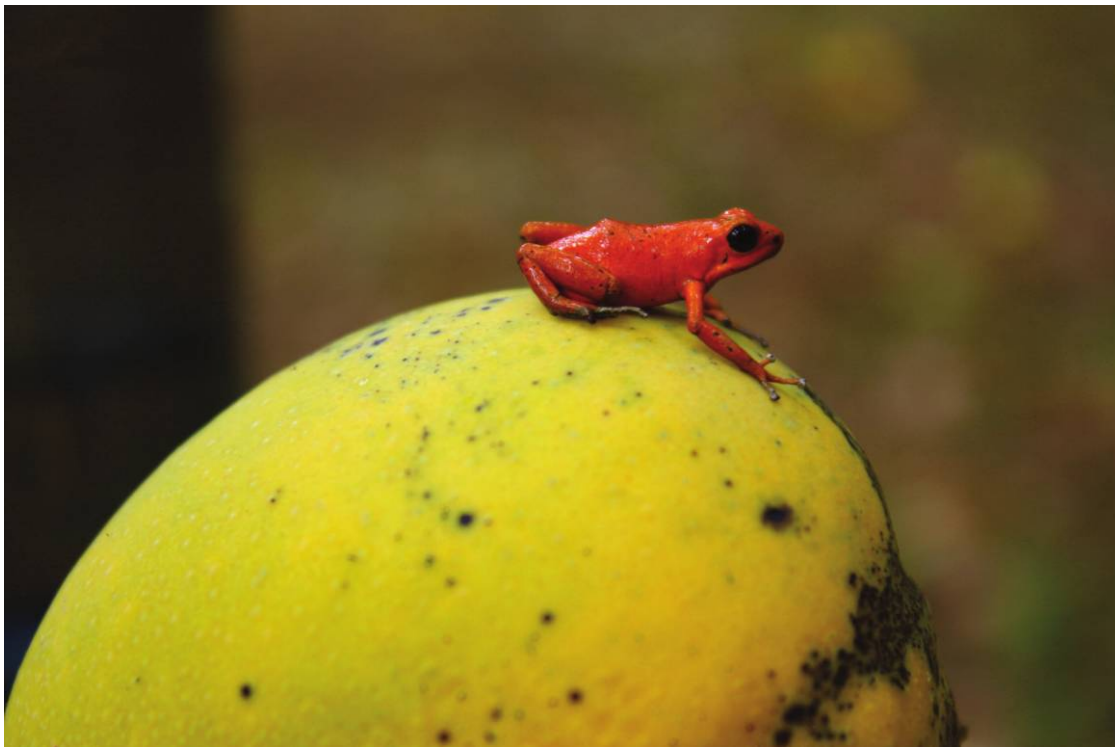
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An adult strawberry poison frog sits atop a mango in the forest of Isla Solarte, Bocas del Toro, Panama. Photograph by Laura R. Crothers.