Poison Frog Colors Are Honest Signals of Toxicity, Particularly for Bird Predators

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ABSTRACT: Antipredator defenses and warning signals typically evolve in concert. However, the extensive variation across taxa in both these components of predator deterrence and the relationship between them are poorly understood. Here we test whether there is a predictive relationship between visual conspicuousness and toxicity levels across 10 populations of the color-polymorphic strawberry poison frog, *Dendrobates pumilio*. Using a mouse-based toxicity assay, we find extreme variation in toxicity between frog populations. This variation is significantly positively correlated with frog coloration brightness, a viewer-independent measure of visual conspicuousness (i.e., total reflectance flux). We also examine conspicuousness from the view of three potential predator taxa, as well as conspecific frogs, using taxon-specific visual detection models and three natural background substrates. We find very strong positive relationships between frog toxicity and conspicuousness for bird-specific perceptual models. Weaker but still positive correlations are found for crab and *D. pumilio* conspecific visual perception, while frog coloration as viewed by snakes is not related to toxicity. These results suggest that poison frog colors can be honest signals of prey unpalatability to predators and that birds in particular may exert selection on aposematic signal design.

Keywords: aposematism, Dendrobatidae, polymorphism, predation, warning coloration, visual modeling.

Introduction

Organisms that are unpalatable to consumers may evolve warning signals to avoid being attacked. Such aposematic signaling is favored by natural selection, as long as the costs of signaling (e.g., increased probability of detection) are offset by its benefits in terms of predation avoidance. Consequently, warning signals and unpalatability are predicted to evolve in concert (Summers and Clough 2001; Sherratt and Beatty 2003; Ruxton et al. 2005). However,

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once these two components of predator deterrence have evolved, the subsequent evolutionary trajectories of both traits are difficult to predict (Speed et al. 2010). Intuitively, one may expect that an increase in the strength of the noxious stimulus (e.g., toxicity) should coincide with greater conspicuousness of the warning signal (e.g., visual contrast), with "nastier" animals "shouting loudest" (Speed and Ruxton 2007). This is because the greater risk of detection and attack for highly conspicuous prey can be compensated for by the stronger predator deterrence induced by high toxicity (Darst et al. 2006). A positive relationship may also emerge from physiological or energetic trade-offs between the two traits (Blount et al. 2009).

While there is some empirical support for these predictions (Summers and Clough 2001; Bezzerides et al. 2007; Cortesi and Cheney 2010), both theoretical arguments and empirical evidence for alternative scenarios exist. In particular, it has been argued that highly toxic prey induce such strong avoidance in predators that conspicuous advertisement traits would not confer additional benefits (Leimar et al. 1986; Speed and Ruxton 2005). Recent work in poison frogs is consistent with this: among three *Epipedobates* species, Darst et al. (2006) found that the two components of predator deterrence could independently contribute to protection, such that increased visual conspicuousness compensated for lower toxicity and vice versa.

Here, we investigate the relationship between toxicity and conspicuousness in the extremely color-polymorphic poison frog *Dendrobates pumilio*. In most of its distributional range in Nicaragua, Costa Rica, and Panama, *D. pumilio* are red dorsally and ventrally with dark blue arms and legs. In the Bocas del Toro Archipelago in northwestern Panama, however, approximately 15 distinct phenotypes occur, spanning the full range of the visual spectrum (Daly and Myers 1967; Myers and Daly 1983;

Summers et al. 2003; fig. 1). Relatively recent geographical isolation (<10,000 years; Anderson and Handley 2002) and incomplete lineage sorting (Brown et al. 2010) suggest a major role for divergent selection on coloration in this species (Summers et al. 1997; Wang and Shaffer 2008; Brown et al. 2010).

Color variation in *D. pumilio* is genetically determined, as evidenced by results from crossing experiments (Summers et al. 2004), the coexistence of multiple morphs in syntopy (e.g., fig. 1), and the observation that morphs retain coloration despite changes in diet (M. E. Maan and M. E. Cummings, unpublished data). In contrast, variation in toxicity has a major environmental component. Like other dendrobatids (Daly et al. 1994; Darst et al. 2005; Saporito et al. 2009), *D. pumilio* obtains alkaloids from a specialized diet of leaf litter arthropods. There are, however, indications for genetically based variation in dendrobatid alkaloid profiles as well (Daly et al. 1987, 2003). While the relative importance of genetic and environmental contributions is critical for reconstructing the evolutionary history of color and toxicity, here we aim to document current patterns of variation as a basis for developing hypotheses about their adaptive value. Specifically, we determine whether the between-population variation in coloration conspicuousness can serve as a reliable indicator of variation in frog toxicity.

A pioneering study by Daly and Myers (1967) already documented substantial toxicity variation among seven different populations of *D. pumilio*, which was apparently unrelated to the variation in coloration. Based on these results, subsequent researchers have assumed that toxicity and coloration are independent in this species. This view has been strengthened by accumulating evidence for the role of *D. pumilio* coloration in intraspecific communication (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008, 2009; Crothers et al. 2011; Richards-Zawacki and Cummings 2011). Here, we reevaluate this conclusion. In comparison with the study by Daly and Myers (1967), we adopt a more sensitive toxicity assay and include additional color morphs. More-
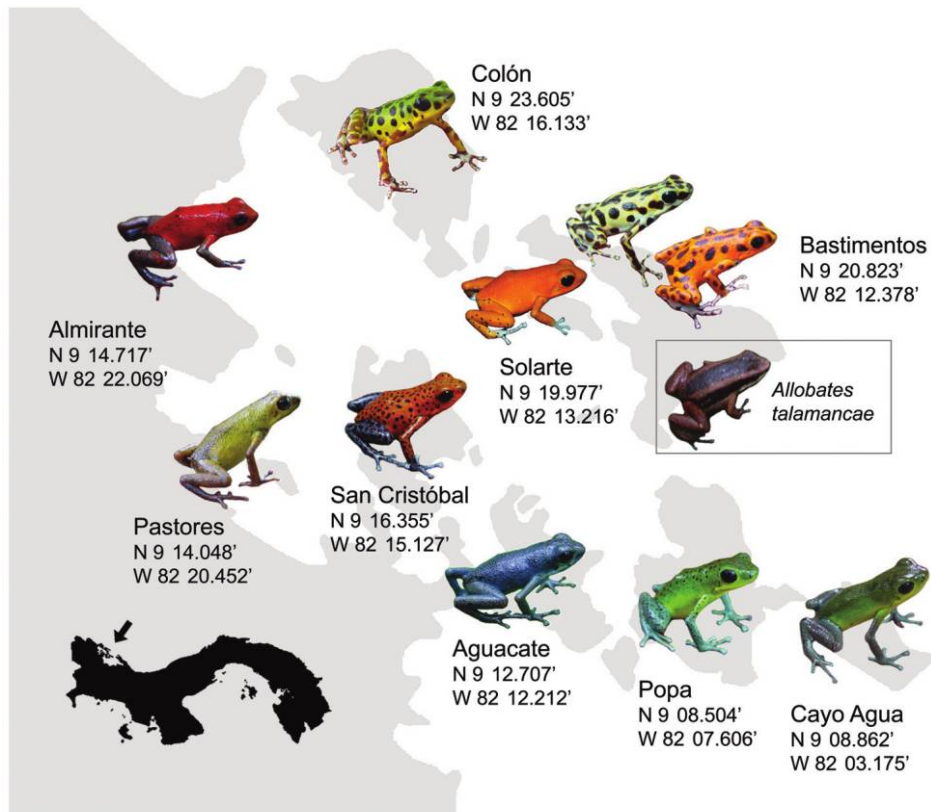

Figure 1: Sampled populations of *Dendrobates pumilio* in the Bocas del Toro Archipelago, Panama. From Isla Bastimentos, two *D. pumilio* color morphs were collected (green and orange), as well as four individuals of the closely related but nontoxic control species *Allobates talamancae.*

over, we use quantitative estimates of frog conspicuousness, including inherent brightness of the signal (total reflectance flux) as well as detectability estimates for conspecifics and three potential predators.

There is extensive evidence that the visual conspicuousness of aposematic signals contributes to their efficacy (Gittleman and Harvey 1980; McGovern et al. 1984; Roper and Redstone 1987; Lindstrom et al. 1999; Darst et al. 2006). However, because of ecological heterogeneity in predator communities and signaling conditions, there is no universal way to maximize conspicuousness (Mappes et al. 2005). Predator-specific perceptual biases in particular may exert divergent selection on aposematic signal design. Frog predators come from widely different taxa (e.g., birds, spiders, snakes, and crabs; Silverstone 1975; Myers and Daly 1976; Formanowicz et al. 1981; Brodie and Tumbarello 1987; Master 1999; Gray and Christy 2000; Gray et al. 2010), representing a variety of visual systems. Birds tend to be tetrachromats (i.e., four photoreceptor classes) and sensitive from ultraviolet (UV) to long wavelengths (Bowmaker et al. 1997). Many higher-order diurnal snakes have three photoreceptor classes and are less sensitive to long wavelengths than birds (Sillman et al. 1997; Macedonia et al. 2009). Crabs are either monochromats or dichromats (Jordão et al. 2007), with sensitivity to long wavelengths intermediate between birds and snakes. As a consequence of this variation, different predator taxa are likely to perceive frog coloration very differently. Additional variation in signal perception emerges from spatial heterogeneity in the visual background against which signals are viewed. Poison frog habitats contain a variety of natural substrates, such as live or dead plant parts, that generate different visual backgrounds. Here, we take variation in both visual backgrounds and predator visual systems into account when evaluating the relationship between frog unpalatability and warning signal conspicuousness. Contrary to the current assumption for this species, these improved methods reveal a strong and positive relationship between toxicity and conspicuousness.

Methods

Frogs

For skin samples, we collected *Dendrobates pumilio* individuals from 10 different color morphs in 9 locations in the Bocas del Toro Archipelago, Panama (fig. 1; June–July 2007). For each color morph, we collected 2 females and 3 males. While these are small sample sizes (because of permit restrictions), previous studies on *D. pumilio* alkaloid profiles indicate that between-population variation is much larger than within-population variation (Saporito et al. 2006, 2010). On Isla Bastimentos, different color morphs occur syntopically on the western side of the island. We collected two of these morphs (green and orange) and treated them as separate samples. At the same site, we also collected four individuals of the closely related but nontoxic species *Allobates talamancae* as controls. Two additional skins were obtained from Aguacate frogs (both males) that were collected for other purposes and died during transport.

Estimates of frog visual conspicuousness were based on reflectance spectrometry (see below). For this we collected an additional sample of frogs that were returned to the collection sites after measurements. Sample sizes were as follows: Solarte, 48; Bastimentos green, 19; Bastimentos orange, 22; Aguacate, 55; San Cristóbal, 5; Almirante, 51; Pastores, 18; Cayo Agua, 15; Popa, 15; Colón, 5; *A. talamancae*, 13.

Toxin Extraction

Frogs were transported to the Bocas del Toro Field Station of the Smithsonian Tropical Research Institute on Colón Island. They were euthanized by applying benzocaine (Orajel, Church and Dwight, Princeton, NJ) to the head and venter. We determined frog weight (to the nearest 0.01 g) and snout-vent length (SVL; to the nearest 0.1 mm). Whole skins were removed and stored in methanol for at least 3 weeks. Methanol extracts from individual frog skins were evaporated under a fume hood, at room temperature, and redissolved in sterile saline (0.2 mL of saline per skin extract).

Toxicity Assay

Dendrobatids, like many other organisms that use toxins for defense, such as birds (Dumbacher et al. 1992), scorpions (Bosmans et al. 2007), spiders (Szelistowski 1985; Gray et al. 2010), and ants (Fritz et al. 1981), use alkaloid compounds that target voltage-gated ion channels (Daly et al. 1980; Daly 1998; Bosmans et al. 2004). Because ion channels are fundamental components of nervous systems across invertebrate and vertebrate taxa, this is a generalized defense. For example, one of the alkaloids found in *D. pumilio* (PTX 251D) is toxic to mice as well as insects (Weldon et al. 2006). While taxon-specific effects of distinct alkaloids cannot be ruled out, here we use a toxicity/irritant assay for one vertebrate group (mice) as a general proxy for the response of natural predators. We assume that subcutaneous injection induces responses that are representative of those generated when predators or parasites attack and/or ingest a frog.

We injected frog skin extracts subcutaneously into sleeping laboratory mice and subsequently recorded the time (in minutes) it took the mice to return to sleep as a mea-

sure of toxicity, where longer latency is assumed to reflect increased toxicity (as in Darst and Cummings 2006; Darst et al. 2006). All toxicity assay experiments were conducted during December 2007 and January 2008 and followed Institutional Animal Care and Use Committee protocols (UT 07092101 and STRI 200715122207).

Mice were obtained from Harlan Laboratories (Indianapolis, IN; outbred strain CD-1, $n = 80$ females) and kept at the Animal Resources Center at the University of Texas at Austin. Mice were injected with 0.2 mL of skin extract (diluted; see below). After return to sleep, mice were euthanized and weighed (to the nearest 1 g; mean $\pm$ SE = 21.9 $\pm$ 0.3 g). Because of the extreme variation in toxicity among populations (see "Results"), and to avoid lethal doses, extracts were diluted (up to 20 ×). Starting dilutions were based on toxicity estimates from Daly and Myers (1967) and adjusted in subsequent trials after observation of the effects. Dosage was calculated as the number of frog skins per kilogram of mouse and ranged from 0.72 to 35.89 (mean $\pm$ SE = 13.24 $\pm$ 1.38). To obtain a "toxicity score," we divided the time until sleep by the dosage and applied log transformation and normalization.

Because of underestimation of lethality in the beginning of the trials, and intrapopulation variation in toxicity, three mice died (Almirante: mice died at 2 × and 3 × dilutions; Solarte: mouse died at 5 × dilution). For one of the Almirante samples, not enough extract remained for another injection, and this frog was excluded from the analysis. One additional sample (from Colón) was excluded because the injection failed. Thus, final sample sizes were $n = 5$ skins for all *D. pumilio* populations except Aguacate (7), Almirante (4), and Colón (4). As controls, we used undiluted skin extracts from *A. talamancae* ($n = 4$) and saline solution ($n = 5$).

Coloration Measurements

To quantify conspicuousness of the *D. pumilio* morphs, we used reflectance spectrometry, habitat spectral irradiance measurements, and visual modeling specific to each viewer. We measured frog and substrate spectral reflectances, $R(\lambda)$, using a StellarNet EPP200C UV-VIS spectrometer, SL-4 Xenon lamp, and an R400-7 reflectance probe positioned at an angle of 90° at a distance of 3 mm from the frog skin, substrate or Spectralon white standard. 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 averaged over the belly and throat regions (two measurements each).

Conspicuousness was evaluated via two methods: (*a*) the total reflectance flux ($\sum_{\lambda=300}^{\lambda=700\,\mathrm{nm}} R(\lambda)$) as a measure of viewer-independent overall brightness and (*b*) viewer-dependent detection models that are taxon-specific: birds, crabs, snakes, and conspecific frogs (see below). In all models, conspicuousness was evaluated in terms of spectral (ΔS) and brightness (ΔL) contrast relative to the background substrate.

Inputs into each visual model include target and background reflectances ($R_t(\lambda)$: *D. pumilio* dorsum or venter; $R_b(\lambda)$: substrate), habitat irradiance ($I(\lambda)$), and taxon-specific photoreceptor absorptance spectra ($A_c(\lambda)$) for each photoreceptor class c of four different viewers. As backgrounds, we used three substrates on which we frequently observed the frogs: (i) tree bark (black brown), (ii) dead leaf litter (brown), and (iii) live *Heliconia* sp. leaf (green). We collected these substrates in the frogs' habitats and measured their reflectance as above (reflectance spectra are given in fig. A1, available in the online edition of the *American Naturalist*). We used a representative habitat irradiance measurement collected in *D. pumilio* habitat on Isla Solarte (for details, see Maan and Cummings 2009; fig. A1). As viewers, we used conspecifics (*D. pumilio* cone absorptance spectra from Siddiqi et al. 2004) as well as three potential predator taxa with very different visual systems. First, we used a dichromatic crab visual model based on *Uca tangeri* long-wavelength-sensitive (LWS) cone absorptance spectra, after correcting for screening oil droplets (Jordão et al. 2007), and electrophysiological measures of a short-wavelength-sensitive (SWS) cone response of *U. thayeri* (Horch et al. 2002). Second, we used a trichromatic snake visual model (coachwhip, *Masticophis flagellum*, Colubridae) with absorptance spectra from Macedonia et al. (2009). Colubridae are some of the most common snake predators in the Bocas del Toro Archipelago (M. E. Maan and M. E. Cummings, unpublished data). Finally, we used two different tetrachromatic bird models. We used a UV-sensitive (UVS) model ($\lambda_{max} = 362$ nm) based on the European starling, *Sturnus vulgaris*, using absorptance spectra after correcting for screening oil droplets from Hart et al. (1998). In addition, we used a violet-sensitive (VS) model ($\lambda_{max} = 409$ nm) based on the pigeon, *Columba livia*, and also correcting for screening pigments (after Bowmaker et al. 1997). Because the two bird models yielded virtually identical results, we present only the UVS model (VS results are given in the appendix, available in the online edition of the *American Naturalist*).

We used a rhodopsin (vitamin A1–based) template (Govardovskii et al. 2000) to generate photoreceptor absorptance spectra for the coachwhip snake with UVS $\lambda_{max} = 362$ nm, SWS $\lambda_{max} = 458$ nm, and LWS $\lambda_{max} = 561$ nm (Macedonia et al. 2009). Model development for the other taxonomic viewers has been reported

elsewhere (*D. pumilio*: Siddiqi et al. 2004; Darst et al. 2006; Maan and Cummings 2009; crab: Cummings et al. 2008; bird: Vorobyev et al. 1998; Cummings et al. 2008).

Each visual model begins with photoreceptor quantum catch, Q_c, for target or background radiance:

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

integrated over 1-nm intervals from 300 to 700 nm.

The quantum catch estimates are then adjusted for the adapting background light environment using the von Kries transformation, such that $q_c = k_cQ_c$ 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 (as in Maan and Cummings 2009).

Subsequently, photoreceptor signal was assumed to be proportional to the logarithm of these adjusted quantum catches (Weber-Fechner laws), such that the contrast between target and background is $\Delta f_c = \ln[q_c(\text{target}) - q_c(\text{background})]$.

We assume that all target detection is subject to photoreceptor noise (ω), which is estimated as a function of the Weber fraction for each cone class (ν) and the relative number of receptor types in the retina (η), where $\omega = \nu/\eta$. The Weber fraction, or Fechner fraction, refers to the constancy by which the difference threshold scales with background intensity under high illumination conditions. We used the measured avian LWS Weber fraction, $\nu = 0.10$ (Maier 1992), for all avian cone classes and used an estimate of $\nu = 0.05$ for *D. pumilio* photoreceptors (as in Siddiqi et al. 2004) as well as all coachwhip photoreceptors. Cone proportions for all vertebrate viewer models were collected from the literature: European starling (Hart et al. 1998): LWS = 0.51, middle-wavelength-sensitive (MWS) = 0.27, SWS = 0.17, UVS = 0.05; higher-order snake (*Thamnophis sirtalis*; Sillman et al. 1997): LWS = 0.85, SWS = 0.10, UVS = 0.05; and *D. pumilio* (Siddiqi et al. 2004): LWS = 0.50, MWS = 0.375, SWS = 0.125. Given the anatomical differences between vertebrate and invertebrate eyes, cone proportion and Weber fraction estimates are not available for the crab eye, and we used electrophysiological noise measurements of the LWS cone class from another invertebrate (honeybee; Vorobyev et al. 2001) as our measurement of photoreceptor noise, $\omega = 0.12$. To ensure that differences in predator-specific estimates of conspicuousness were not driven by noise estimation, we compared our results to a run of all the models applying a constant photoreceptor noise estimate for each predator's photoreceptors ($\omega = 0.12$). This yielded dif-

ferent conspicuousness estimates but did not change the results qualitatively (see appendix).

The next stage of the viewer models assumes that target detection is evaluated as both color (spectral) and brightness (luminosity) contrast, ΔS and ΔL, respectively. Color contrast estimates (ΔS) were evaluated according to visual system (dichromat, trichromat, or tetrachromat), where U, S, M, and L represent the UVS, SWS, MWS, and LWS cone classes, respectively. The equations are as follows. For dichromat (ΔS_{crab}),

$$\Delta S = \sqrt{[(\Delta f_L - \Delta f_S)^2/(\omega_S^2 + \omega_L^2)]}.$$

For trichromat ($\Delta S_{\text{snake or frog}}$),

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

For tetrachromat (ΔS_{avian}),

$$\Delta S = \{[(\omega_U\omega_S)^2(\Delta f_L - \Delta f_M)^2 + (\omega_U\omega_M)^2(\Delta f_L - \Delta f_S)^2$$
$$+ (\omega_U\omega_L)^2(\Delta f_M - \Delta f_S)^2 + (\omega_S\omega_M)^2(\Delta f_L - \Delta f_U)^2$$
$$+ (\omega_S\omega_L)^2(\Delta f_M - \Delta f_U)^2 + (\omega_M\omega_L)^2(\Delta f_S - \Delta f_U)^2]$$
$$/[(\omega_U\omega_S\omega_M)^2 + (\omega_U\omega_S\omega_L)^2 + (\omega_U\omega_M\omega_L)^2 + (\omega_S\omega_M\omega_L)^2]\}^{1/2}.$$

Brightness contrast (ΔL), that is, the ability to discriminate target from background in the luminance channel, is assumed to be governed by the LWS cone class alone in many terrestrial organisms such as birds (Maier and Bowmaker 1993) and honeybees (Spaethe et al. 2001; Théry and Casas 2002). Hence, signal-to-noise estimates in the luminance channel were evaluated with $\Delta L = |\Delta f_{\text{LWS}}/\omega_{\text{LWS}}|$ in all viewer models.

For each viewer/background combination, we calculated the relative conspicuousness of the 10 *D. pumilio* populations in terms of brightness contrast (ΔL), spectral contrast (ΔS), and overall conspicuousness. Overall conspicuousness represents the combined contrast of both ΔL and ΔS and is evaluated as the Euclidean distance from the origin in perceptual space with brightness contrast (ΔL) on the X-axis and spectral contrast (ΔS) on the Y-axis (as in fig. 3).

Data Analysis

All analyses were conducted in R (R Development Core Team 2009). Variation in toxicity between populations, and between males and females, was analyzed using generalized linear mixed-effects models (lme4 package). To calculate repeatability (following Lessells and Boag 1987), skin extracts of 11 frogs were injected twice. These trials were

also included in the glm analysis, where pseudoreplication was controlled for by including a factor for "individual" as a random effect. Significance of fixed effects was determined using χ^2 tests comparing alternative models. Two-way comparisons of toxicity between populations were done with Tukey's post hoc HSD tests, where pseudoreplication was avoided by using the average toxicity scores of repeated individuals.

Analysis of relationships between frog coloration and toxicity were conducted at the population level, using population means for toxicity scores and coloration measures. We used Pearson correlation and generalized linear models. Several studies have shown rapid phenotypic evolution and incomplete lineage sorting in *D. pumilio* (Wang and Shaffer 2008; Brown et al. 2010). Therefore, analyses were not adjusted for phylogenetic dependence.

Results

Population Variation in Toxicity

After injection, mice returned to sleep after a latency of 11–224 min, ranging from (mean $\pm$ SE) 39 $\pm$ 6 min for saline controls to 102 $\pm$ 18 min for (diluted) Solarte extracts. Relatively mild symptoms of discomfort, such as elevated levels of grooming, were observed in the majority of experiments. More serious symptoms (piloerection, uncontrolled movements, loss of balance, convulsions) were observed in about half of all experiments but never in controls (i.e., saline and *Allobates talamancae* extracts).

Repeatability of toxicity score was 0.74 (Pearson correlation $r = 0.75$, $P = .008$, $R^2 = 0.56$; based on $n = 11$ samples that were used twice). There were highly significant differences in toxicity scores between *Dendrobates pumilio* populations ($\chi^2 = 94.13$, df = 9, $P \ll .001$; fig. 2). The dosage-corrected time until sleep was more than 40-fold higher for the most toxic population, Solarte, compared to that of the least toxic population, Colón. Out of 10 populations, 7 were significantly more toxic than the saline control and 6 were more toxic than the *A. talamancae* control.

There were no significant differences in toxicity between male and female frogs ($\chi^2 = 1.38$, df = 1, $P = .24$), and there was no effect of frog size (weight: $\chi^2 = 0.22$, df = 1, $P = .64$, SVL: $\chi^2 = 0.54$, df = 1, $P = .46$).

Toxicity and Coloration Brightness

We evaluated the relationship between frog toxicity and overall brightness, a viewer-independent measure of conspicuousness (reflectance flux, ΣR). We found that toxicity score was positively correlated with the total brightness of dorsal coloration (Pearson $r = 0.78$, $P = .0078$; fig. 2)

but not ventral coloration ($r = 0.42$, $P = .18$; fig. A2 in the online edition of the *American Naturalist*).

Viewer-Specific Perception of Frog Coloration

We then evaluated the visual contrast of frog coloration for four different viewers (frog, crab, bird, and snake) and three different background substrates (bark, leaf litter, and *Heliconia*). We found that the perception of visual contrast was significantly different between different viewers, for both ventral and dorsal coloration ($F_{2,105} > 20.88$, $P \ll .0001$; e.g., fig. 3). Background substrate did not significantly affect the among-viewer variation in visual contrast ($F_{2,105} < 1.05$, $P > .35$). Detailed results on color and brightness contrast for all viewers and all backgrounds can be found in the appendix.

Toxicity and Viewer-Dependent Conspicuousness

For each viewer-background combination, we determined the relationship between frog overall conspicuousness (Euclidean distance of color and brightness contrast in color space; e.g., fig. 3) and toxicity. Regarding dorsal conspicuousness (fig. 4), we found all relationships to be positive: more toxic frogs generated greater visual contrast, and this held true whether using species-specific photoreceptor noise estimates or a constant noise estimate ($\omega = 0.12$; see appendix). For bird viewers in particular, all relationships were strong and highly significant (*Heliconia*: $r = 0.96$; leaf litter: $r = 0.92$; bark: $r = 0.85$; $P < .001$ for overall conspicuousness [fig. 4]; $P < .004$ for all brightness contrast [ΔL] measures [fig. A4 in the online edition of the *American Naturalist*]; and $P < .04$ for the color contrast [ΔS] measure against a *Heliconia* background [fig. A5 in the online edition of the *American Naturalist*]), independent of the VS/UVS visual system (VS system data shown in table A2 in the online edition of the *American Naturalist*). This indicates that regardless of the visual background, frog conspicuousness as perceived by birds is a good predictor of toxicity.

For the crab visual system, frog overall conspicuousness against a leaf litter background was significantly correlated with toxicity ($r = 0.64$, $P = .046$), with bark backgrounds yielding a statistical trend ($r = 0.61$, $P = .064$) but no relationship for *Heliconia* backgrounds ($P = .20$). For the snake visual system, none of the relationships were significant (all $P = .13$). Finally, overall conspicuousness for the frog visual system showed positive trends for correlations with toxicity, for each of the three background substrates (all $r > 0.61$, $P < .06$). Interestingly, the strength of the relationship between toxicity and taxon-specific overall conspicuousness or brightness contrast (ΔL) was
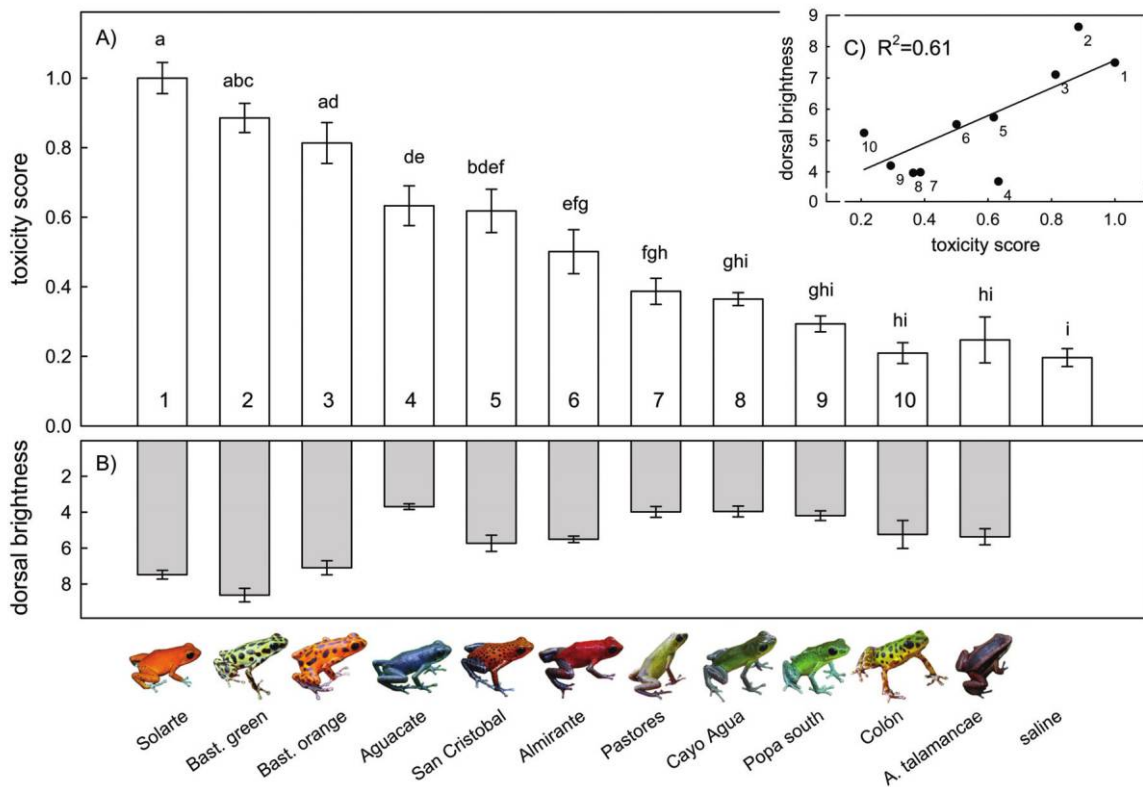
Figure 2: Toxicity scores and coloration brightness in *Dendrobates pumilio*. *A*, Open bars indicate toxicity scores with standard errors. Different letters above the bars indicate statistically significant differences; numbers are population numbers referred to in *C* and in subsequent figures. *Allobates talamancae* (a closely related Dendrobatid frog) and saline solution served as toxicity measure controls. *B*, Gray bars indicate the overall brightness of dorsal coloration (total reflectance flux, ΣR, in arbitrary units) for the 11 frog taxa. *C*, The inset gives the correlation between toxicity and dorsal brightness for the 10 *D. pumilio* populations. Numbers refer to the population labels in *A*.

stronger for avian viewers than for *D. pumilio* conspecific viewers (fig. 4).

Contrary to this pattern, ventral conspicuousness was not related to frog toxicity for any of the viewers, on any of the background substrates (all $P > .5$; fig. A3 in the online edition of the *American Naturalist*). The difference between dorsal and ventral coloration in predicting toxicity was statistically significant for the bird visual system on all background substrates (all $F_{2,16} > 5.58$, $P < .02$), as well as for the crab visual system on a leaf litter background ($F_{2,16} = 4.25$, $P = .033$; all other $P > .08$).

Discussion

Diversity in aposematic signal design is a poorly understood phenomenon. Our study of a color-polymorphic poison frog contributes three main insights. First, we find that the extreme color diversity of *Dendrobates pumilio* is mirrored by substantial variation in toxicity. Second, we

show that these two components of predator deterrence are correlated, with toxic frogs displaying more conspicuous coloration. Third, our visual ecology approach implicates birds as potentially important frog predators because they show the strongest predictive relationships between toxicity and viewer-specific conspicuousness estimates—surpassing conspecifics as well as the other two predators considered here. Together, these results suggest that both predator selection and alkaloid availability influence frog coloration and that environmental heterogeneity in these two factors may generate diversity in aposematic signal design.

Honest Signaling across Diverse Morphs and Populations

Across 10 diversely colored populations of *D. pumilio*, we found that the conspicuousness of frog coloration was significantly positively correlated with frog toxicity. This
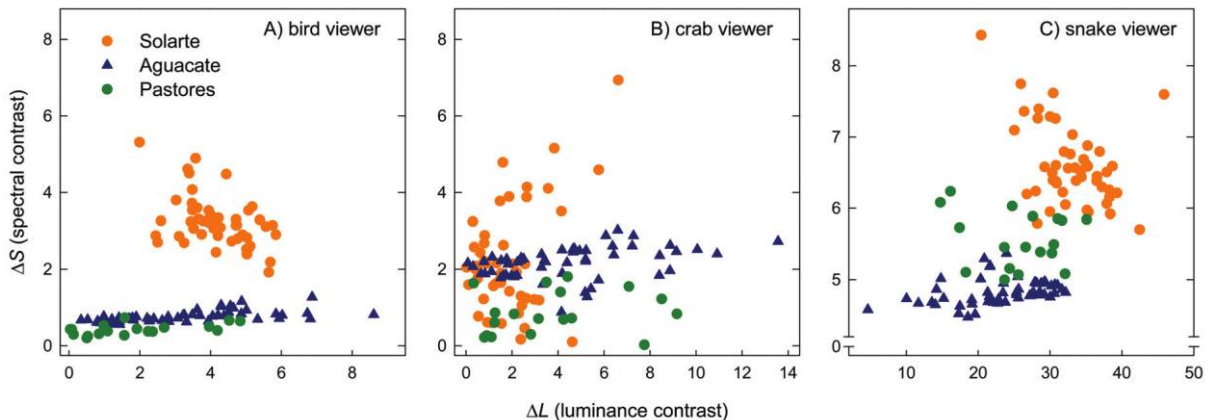
Figure 3: Illustration of the perceptual differences between potential predator taxa in species-specific color space. Plots show the estimated brightness and color contrast generated by the dorsal coloration of individuals from three *Dendrobates pumilio* populations (Solarte [orange], Aguacate [blue], and Pastores [green]), viewed against a *Heliconia* background, in the visual systems of birds, crabs, and diurnal snakes. Each dot represents reflectance spectra measured from an individual frog. The relative conspicuousness of the different frog morphs differs by viewer. For example, while crabs tend to perceive greater color contrast (ΔS) for blue frogs compared to green frogs, the difference is reversed for the snake visual system and negligible in the bird visual system.

relationship was found for both inherent measures of conspicuousness (i.e., total reflectance flux or reflectance brightness; fig. 2) and viewer-specific estimates of conspicuousness (fig. 4). Frog conspicuousness may thus serve as a reliable predictor of toxicity. Previous research has addressed the possibility that "honest" warning signals emerge from physiological trade-offs, mediated by limited resources (Bezzerides et al. 2007; Blount et al. 2009). This hypothesis predicts that *D. pumilio* populations are resource limited to various extents, a prediction that could be tested by comparing the strength of intrapopulation correlations between coloration and toxicity. Little is known about the physiology of both toxin sequestration and amphibian coloration. The extreme diversity in *D. pumilio* coloration, involving a variety of pigments and structures, together with the diversity in alkaloid compounds, suggests that any intrinsic trade-off underlying their association would have to generalize across various physiological pathways. Alternatively, or in addition, honesty in warning signals may be driven by the costs and benefits of conspicuousness in terms of predation risk. Conspicuous signals increase the probability of detection by predators, but they also enhance predator learning and memory (Gittleman and Harvey 1980; McGovern et al. 1984; Roper and Redstone 1987; Lindstrom 1999; Darst et al. 2006). Because toxicity contributes to predator learning as well (Darst et al. 2006), highly toxic frogs induce stronger and more persistent avoidance in predators and are therefore less likely to be attacked after detection or ingested after attack. This means that highly toxic frogs

can take advantage of the enhanced avoidance induced by conspicuous colors, while less toxic frogs will suffer the costs without reaping the benefits.

While we should be cautious in extrapolating our toxicity results to predator taxa beyond mammals, our investigation suggests a 40-fold difference between the most toxic frogs (Isla Solarte) and the least toxic frogs (Isla Colón). We are not aware of any other poison frog species with such an extreme variation in toxicity. We did not find toxicity differences between males and females, contrary to Saporito et al. (2010). This is likely due to our sampling design with relatively few individuals per population. Our estimates are largely consistent with those of Daly and Myers (1967), except that we found substantially higher toxicity in Solarte frogs. We found their toxicity to be comparable to Bastimentos frogs, instead of five times lower as in the assay of Daly and Myers (1967). Whether this difference is due to sampling effects or ecological changes in this population is unclear. Temporal variation in alkaloid profiles has been documented in this species, but unfortunately historical data for the Solarte population are not available (Saporito et al. 2007*c*). Given that the frogs in this population are also the most conspicuously colored, the present toxicity estimate fits the overall pattern of a positive relationship between toxicity and warning signal strength. Importantly, however, this pattern persists when the Solarte frogs are excluded from the analysis (see table A6 in the online edition of the *American Naturalist*).

Our results in *D. pumilio* are different from the observation by Darst et al. (2006) of a decoupled relationship
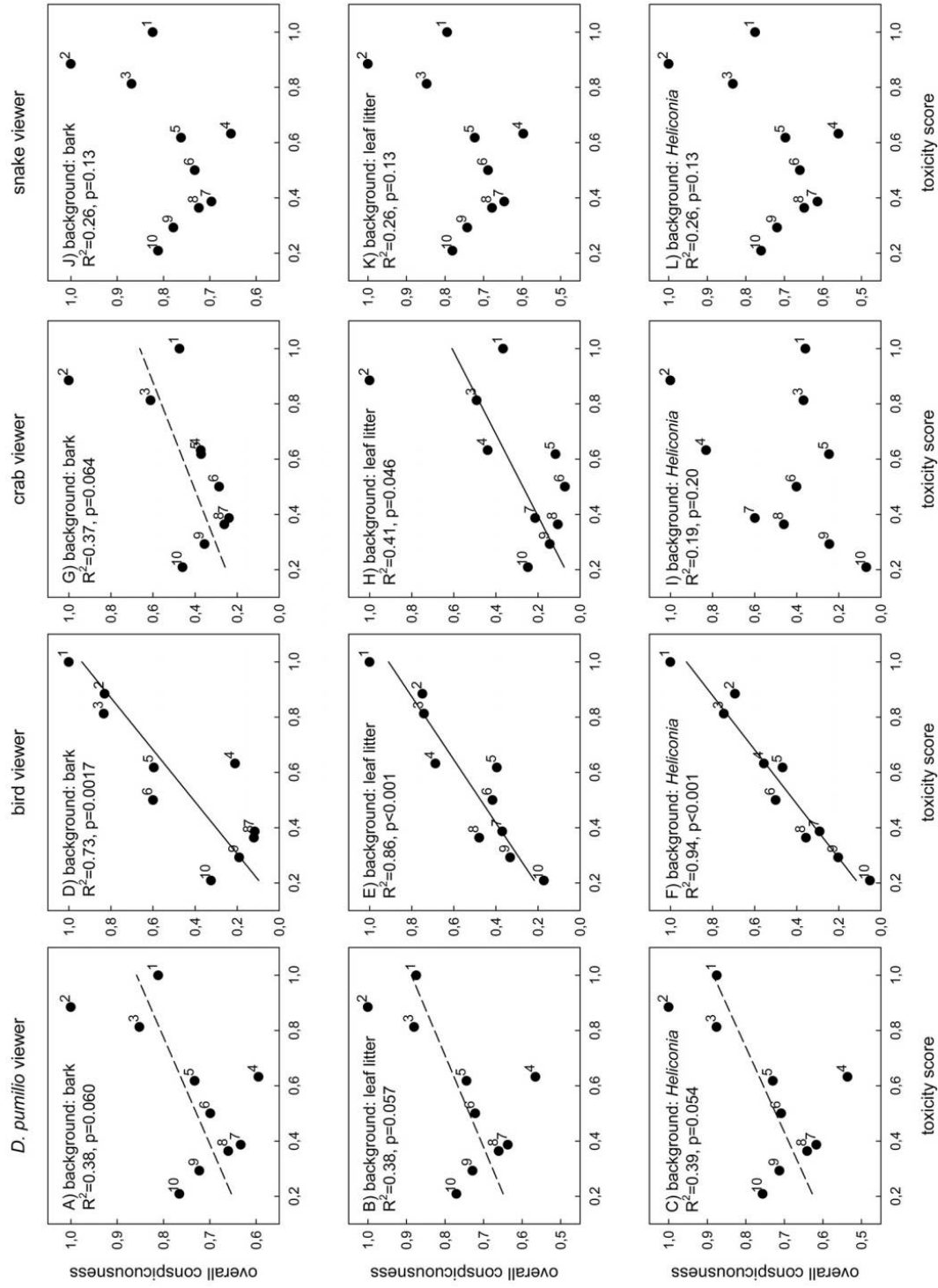
Figure 4: Frog toxicity in relation to visual conspicuousness of dorsal coloration. Plots show the relationship between frog toxicity and the visual contrast of dorsal coloration, as estimated for four different viewers (conspecifics [*A–C*] and three potential predators: bird [*D–F*], crab [*G–I*], and snake [*J–L*]) and three different background substrates (bark [*A, D, G, J*], leaf litter [*B, E, H, K*], and *Heliconia* [*C, F, I, L*]). Solid lines are statistically significant relationships; broken lines indicate statistical trends. Each symbol represents one *Dendrobates pumilio* population, where numbers refer to population labels in figure 2.

between toxicity and conspicuousness in three *Epipedobates* species. However, these *Epipedobates* species coexist with successful Batesian mimics. As a result, optimal conspicuousness and toxicity depend on the abundance of these mimics, and the relationship between the two may be complex. In the *D. pumilio* system, predator responses should be driven by experiences with specific *D. pumilio* morphs alone and not by the relative abundances of models and mimics.

Although most of the theory on aposematic signal evolution deals with the initial origin of warning signals, some recent models have identified a number of factors that influence signal strength, such as prey density, physiological costs of display and defense, and the probability of mortality after attack (e.g., Speed and Ruxton 2007; Speed et al. 2010). To evaluate whether *D. pumilio* fits these scenarios, more ecological data are required. In particular, it is unclear to what extent frog toxicity is constrained by the availability of alkaloids in their environment. This is important because the mechanism underlying toxicity variation will determine its evolvability and thereby the potential coevolution with signal conspicuousness. There is ongoing discussion in the literature regarding the relative importance of genetic and environmental factors in determining poison frog toxicity. A genetic contribution is suggested by the observation that sympatric species can have different alkaloid profiles (Daly et al. 1987) and that certain compounds can be synthesized (or modified) by the frogs themselves (Daly et al. 2003). On the other hand, experiments show that alkaloids are obtained from the diet (Daly et al. 1994; Saporito et al. 2009), and several arthropods have been identified as alkaloid sources (e.g., Daly et al. 2002; Saporito et al. 2004, 2007b; Clark et al. 2005).

Toxicity variation in *D. pumilio* may be driven by three potential mechanisms. First, the islands of the Bocas del Toro Archipelago may differ in alkaloid availability, as a result of heterogeneity in arthropod communities, or in the vegetation from which the alkaloids ultimately derive. Second, alkaloid availability may be homogeneous, but frog populations may differ in foraging strategy, for example, selecting prey of different alkaloid content in different populations. Third, frog populations may differ in the ability to modify or synthesize alkaloids. While we lack direct evidence for any of these possibilities, our study supports a dominant environmental component. We found that two distinct color morphs collected in exactly the same location, orange and green frogs from Bastimentos, exhibit indistinguishable levels of toxicity (fig. 2; see also Daly and Myers 1967). This suggests a major role of environmental variation, most likely prey availability. Consistent with this, a study by Saporito et al. (2006) on the same island reported very similar alkaloid profiles

among individuals collected at the same time and place but differences between seasons and spatial locations. Differences among populations in selective foraging seem unlikely, given that captive frogs readily accept nontoxic prey items (crickets, termites, and fruit flies) and survive and breed for many years on such a diet. Between-population variation in the ability to sequester or modify alkaloids remains to be investigated in this system.

Using Visual Ecology to Infer Probable Predators

The positive relationship between toxicity and conspicuousness was significant for viewer-independent brightness (fig. 2) and many of the taxon-specific visual contrast estimates (overall conspicuousness [fig. 4], brightness contrasts [fig. A3], and color contrast [fig. A4]). We found a particularly strong relationship for the bird visual system, even stronger than for conspecifics and for other potential predators. This not only suggests that frog colors evolve under natural selection but also implicates birds as important predators. In support of this hypothesis, field observations have identified a large number of frog-eating bird species (Poulin et al. 2001), and experiments with artificial frog models have found that birds account for a large number of attacks and respond to color differences between models (Saporito et al. 2007a; Noonan and Comeault 2009).

Documenting predation in the wild is notoriously difficult, even more so for aposematic prey with extremely low attack rates. We suggest that comparative visual ecology, as adopted here, may be a useful approach in identifying probable predators in other systems as well. Yet, while bird predation may be largely responsible for the observed correlation between coloration and toxicity, it should be noted that avian predators are likely to rely heavily on visual cues for predation, whereas the relative importance of visual predation strategies for diurnal snakes or crabs is less known. Indeed, the lack of a relationship between toxicity and visual conspicuousness as perceived by snake viewers may be a function of snake olfactory abilities to detect their prey and assess palatability. Moreover, specific alkaloids may vary in the extent to which they deter specific predator taxa that use chemical rather than visual cues for prey detection (Weldon et al. 2006).

In contrast to the differences between predator taxa, we did not find a major effect of variation in visual backgrounds on the relationship between toxicity and conspicuousness. This suggests that differences between frog populations in microhabitat use may not explain a large component of color variation, unless different habitats harbor different predator communities (Endler and Mappes 2004; Mappes et al. 2005).

Dorsal Signals Driven by Natural Selection?

While both dorsal and ventral body areas show extreme color variation between *D. pumilio* populations (Summers et al. 2003), only dorsal coloration predicted toxicity. This result strengthens the case for predator selection, given that the frog dorsum will be most visible to predators. It also suggests that ventral coloration is subject to other selective pressures because ventral coloration is often different from dorsal coloration, and the recent divergence makes selective neutrality unlikely (Brown et al. 2010). It is possible that sexual selection by female choice or male-male competition plays a role. During conspecific social interactions, male *D. pumilio* adopt an upright position that exposes their ventral body areas. Ventral coloration tends to be brighter than dorsal coloration in all our study populations (M. E. Maan and M. E. Cummings, unpublished data), and coloration brightness affects both male aggression (Crothers et al. 2011) and female choice (Maan and Cummings 2009). While the behavioral experiments conducted to date did not reveal sexual selection on ventral coloration, dedicated experiments that specifically address ventral coloration have yet to be conducted.

With regard to dorsal coloration, natural selection by predators and sexual selection by female choice are likely to interact. First, female choice may contribute to locally adapted coloration by favoring specific levels of conspicuousness. Consistent with this hypothesis, the most toxic population (Solarte) provides strong evidence for sexual selection on coloration brightness, in terms of female preference behavior as well as sexual dimorphism (Maan and Cummings 2009). Second, within a range of conspicuousness levels, female choice may select for specific colors. Depending on viewer and background, different hues can generate similar visual contrast (e.g., cf. populations 5 [San Cristóbal, red] and 10 [Colón, green] in fig. 4*A*–4*C*). This may provide opportunities for sexual selection to drive some of the observed variation in hue, without being checked by natural selection. Color-mediated female preferences have been documented in several populations of *D. pumilio* (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008), but it is unclear whether these preferences are constrained by local predator-specific perception of frog conspicuousness.

Conclusion

We have shown that the color diversity observed in *Dendrobates pumilio* is tightly linked to variation in toxicity. To test whether coloration adapts to toxicity or vice versa, future studies should address the environmental and physiological constraints on both traits. We have also shown that the perception of frog coloration is taxon specific, and

that birds in particular may exert strong selection on poison frog warning coloration. To conclude, we suggest that the polymorphic colors of *D. pumilio* are honest signals of toxicity, maintained by the potentially complex interactions between predator selection, alkaloid sequestration, and the requirements of intra- and interspecific communication.

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Dendrobates pumilio from Isla Bastimentos. Photograph by Martine E. Maan.