

A mechanism for diversity in warning signals: Conspicuousness versus toxicity in poison frogs

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Edited by David B. Wake, University of California, Berkeley, CA, and approved February 26, 2006 (received for review January 24, 2006)

Many animals advertise their chemical defense to predators with conspicuous coloration and unpalatability, but little is known about the information in these signal elements. To effectively avoid predation, is it more advantageous to invest in increased conspicuousness or greater noxiousness, or to allocate equally to both signal modalities? Using natural variation among poison frog species measured with spectral reflectance and toxicity assays, we tested the relative importance of warning signal components with predator-learning and avoidance experiments. We demonstrate that closely related species use alternative strategies: increasing either conspicuousness or toxicity affords equivalent avoidance by predators and protection to nontoxic mimic species. These equally effective predator avoidance tactics demonstrate different aposematic solutions for two potentially costly signal components, providing a mechanism for natural diversity in warning signals.

aposematism | chemical defense | predation

Escaping pr

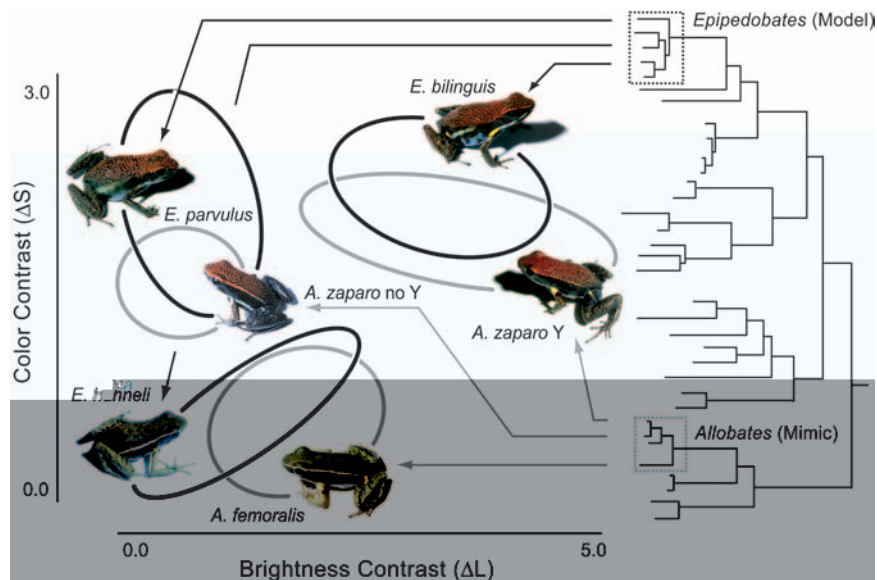


Fig. 1. Conspicuousness of poison frog species as viewed by a potential avian predator. *E. bilinguis* ($n = 16$) and sympatric *A. zaparo* Y ($n = 15$) have a mostly red granular dorsum with yellow blotches in axilla and groin regions (red + yellow); *E. parvulus* ($n = 16$) and *A. zaparo* no Y ($n = 12$) have a red dorsum but lack the yellow regions (red only); and *E. hahneli* ($n = 10$) and *A. femoralis* ($n = 11$) have a dark brownish dorsum with the yellow blotches in the axilla and groin (yellow only). The y axis is color contrast (ΔS = spectral discrimination), and the x axis is brightness contrast (ΔL = long wavelength sensitivity cone contrast) as computed using frog color radiances in an avian visual model (18, 19). Conspicuousness is based on dorsal internal contrast comparing head, back, axilla, and groin areas to side body accounting for the relative body area for each color patch. Ellipses show 95% confidence intervals for each species; the ellipse of each mimic (gray) overlaps with each respective model species (black). Phylogeny of Dendrobatidae is adapted from ref. 8.

from injection of the less toxic species skin extracts were not significantly different from one another ($Z_{biling\ i-hahneli} = -1.571$, $P = 0.116$). Injection of *Allobates zaparo* Y, *A. zaparo* no Y, and *Allobates femoralis* skin extract caused no adverse reaction (no difference among reactions from *A. zaparo* and *A. femoralis* skin extracts and saline control injections; A OVA, $P = 0.535$). These results demonstrate variation in chemical defense among *Epipedobates* species and confirm the absence of alkaloids in *Allobates* (3, 14, 20), suggesting an adaptive function for color pattern convergence (Fig. 1).

Variation in Conspicuousness. Darst and Cummings (3) demonstrated color pattern convergence by the two color morphs of *A. zaparo* (Y and no Y) to geographically localized models (*E. bilinguis* in the north and *E. parvulus* in the south). By converging on a toxic model's color pattern, the mimic is ultimately adopting the model's degree of visual salience (conspicuousness). We evaluated conspicuousness of the three color patterns (red only, yellow only, and red + yellow) from a bird's eye view using an avian visual model that evaluated conspicuousness as a combination of color and brightness contrast (18, 19). We calculated conspicuousness as the dorsal internal contrast comparing head, back, axilla, and groin areas to side body accounting for the relative body area for each color patch (Fig. 1). Hence, both color and brightness contrast (ΔS and ΔL , Fig. 1) are weighted functions of the relative body area for each color patch, producing a measure of whole body conspicuousness that is more appropriate than single patch comparisons (21). Total conspicuousness was evaluated as vector distance in a perceptual space (i.e., Euclidean distance; see Table 1). We found that conspicuousness varies across species, and that each nontoxic *Allobates* has converged on the conspicuousness of a toxic sympatric *Epipedobates* species (Fig. 1; Kruskal-Wallis test; $Z_{parvulus-apar\ no\ Y} = -1.319$, $P = 0.187$; $Z_{biling\ i-apar\ o\ Y} = -0.184$, $P = 0.854$; $Z_{hahneli-femoralis} = 0$, $P = 1.00$). *E. bilinguis*, with both red and yellow color elements, is the most conspicuous of the toxic frogs, followed by *E. parvulus* and *E. hahneli*, each with single color

elements, which do not differ significantly from one another in conspicuousness (Fig. 1; $Z_{biling\ i-parvulus} = -4.336$, $P < 0.001$; $Z_{biling\ i-hahneli} = -4.005$, $P < 0.001$; $Z_{parvulus-hahneli} = -1.606$, $P = 0.108$).

We found that the most toxic species, *E. parvulus* (red only), is not the most conspicuous, whereas the most conspicuous species, *E. bilinguis* (red + yellow), shows only moderate toxicity. *E. hahneli* (yellow only), displays moderate levels of both signal components (Fig. 1). This unexpected pattern of variation allows for controlled comparisons of the relative importance of conspicuousness and toxicity for warning signal effectiveness (i.e., *E. bilinguis* and *E. hahneli*, which differ significantly in conspicuousness but not in toxicity; and *E. parvulus* and *E. hahneli*, which differ significantly in toxicity but not in conspicuousness). Interestingly, the color patterns of all three brightly colored toxic species are mimicked by a nontoxic *Allobates*, suggesting Batesian mimicry (Fig. 1).

Effectiveness of Aposematic Signal Components for Avoiding Predation. We examined the relative contributions of conspicuous coloration and unpalatability to escaping predation with two measures: speed of avoidance learning and degree of avoidance after learning. Predator learning experiments were conducted by using live frogs and naive chicken predators in which predators were exposed to one of three learning stimuli in a series of learning trials: (i) high conspicuousness, moderate toxicity (*E. bilinguis*); (ii) moderate conspicuousness, high toxicity (*E. parvulus*); or (iii) moderate conspicuousness, moderate toxicity (*E. hahneli*). We found that speed of learning was mediated by toxicity. Predators learned most quickly on the most toxic frog (Fig. 2; $n =$ six chicks per treatment; *E. parvulus* mean learning slope, 40.33 ± 8.11 ; *E. bilinguis*, 18.04 ± 7.4 ; *E. hahneli*, 16.60 ± 2.36 ; $Z_{parvulus-bilinguis} = 1.992$, $P = 0.046$; $Z_{parvulus-hahneli} = 2.005$, $P = 0.045$). Toxic frogs were rejected with no harm to the predator, suggesting that greater toxicity confers protection through increased unpalatability. Our results also showed that increased conspicuousness had no effect on learning speed: predators learned at similar rates on highly and moderately

conspicuous frogs of similar toxicity ($Z_{biling\ i\ -hahneli} = 0.7488, P = 0.810$).

Although the first measure, speed of avoidance learning, is important for protection from predation, the ultimate determination of advantage is the second measure, the degree to which predators avoid aposematic individual

conspicuousness functional Batesian mimicry? We found that the mimics successfully deceive predators. Chick predators trained with each model avoided the respective mimic as well, an empirical confirmation of Batesian mimicry by not one but two closely related species in a distantly related clade. Mimics of either the more toxic or more conspicuous model received the high degree of avoidance afforded to their respective model (Fig. 3; *A. apa o* no Y, 0.08 ± 0.01 , $Z_{pa l - apa o no Y} = 1.046$, $P = 0.295$; *A. apa o* Y as stimulus, 0.15 ± 0.03 , $Z_{biling i - apa o Y} = -0.646$, $P = 0.518$). Accordingly, the mimic of the moderately conspicuous and moderately toxic frog received the same moderate degree of avoidance afforded to its model, significantly less than that conferred to *A. apa o* Y and no Y (Fig. 3; *A. femo ali*, 0.46 ± 0.06 ; $Z_{femo ali - apa o Y} = -2.802$, $P < 0.005$; $Z_{femo ali - apa o no Y} = -2.807$, $P < 0.005$).

Discussion

Our results uncover different aposematic solutions to effectively avoid predation that take advantage of the relative benefits of toxicity and conspicuousness. Predators learn more quickly to avoid highly versus moderately toxic prey, whereas an increase in greater conspicuousness does not increase the speed of learning. However, enhancing the complexity of the prey environment with both conspicuous and cryptic prey, the advantage of increased conspicuousness becomes apparent. The benefit of increasing conspicuousness, independent of toxicity, is a significant gain in protection from predation, suggesting that conspicuous coloration helps predators distinguish toxic from palatable prey. We find that poison frog species use different combinations to achieve the same effect; equal protection is achieved with a combination of moderate toxicity and high conspicuousness as with high toxicity and moderate conspicuousness. Our findings reveal equally effective aposematic strategies, providing a mechanism for natural diversity in warning signals (Fig. 4).

Aposematism succeeds when predators associate conspicuousness with unprofitability, and in dendrobatid frogs, multiple origins of conspicuousness are correlated with multiple acquisitions of toxicity (8). During origins of aposematism (evolutionary transitions from cryptic to aposematic signals), a positive correlation between conspicuousness and the strength of defense is predicted (2) and has been reported (25). Our empirical data suggest that after this correlation is achieved, degree of con-

spicuousness and level of defense may become dissociated and adjusted independently. We find that conspicuousness and unpalatability are decoupled: *E. biling i* and *E. hahneli* differ significantly in conspicuousness but not in toxicity, whereas *E. pa l* and *E. hahneli* differ significantly in toxicity but not in conspicuousness (Fig. 4). Our results suggest the hypothesis of a tradeoff between the two components of aposematism for effectively and efficiently escaping predation. Theoretical work has anticipated cross-compensation between potentially costly unprofitability and bright coloration, predicting that optimal investment in secondary defense will diminish when more cost-effective conspicuousness evolves as primary defense (11, 26). There will, however, be constraints in how signal components can be adjusted, particularly in cases of Müllerian mimicry and limited genetic variability. Theor

may depend upon the availability of alternative, nontoxic prey, which generates predictions that are testable in the field.

Warning coloration would initially be favored only after the acquisition of chemical defense, suggesting that conspicuous mutants arise from defended cryptic species (30, 31). Few aposematic forms, therefore, will be selected against because of their conspicuousness and rarity (32). Interestingly, in poison frogs, the benefits of signaling may be conferred by individual selection; we found that $79.24\% \pm 1.78$ of frogs sampled survived the attack, i.e., were tasted by the chick and promptly rejected with no harm to the frog ($n = 62$ sampled frogs). Hence, individuals with novel combinations of the two signal components are able to survive and reproduce, providing greater evolutionary lability in aposematic signals.

Our results demonstrate alternative strategies for combining toxicity and conspicuousness, suggesting that decoupling warning signal components enables effective a

brightness (ΔL) contrast by comparing head, back, axilla, and groin areas to flanks (side body) accounting for the relative body area of each color patch. We used photographs of model frogs viewed from above to estimate the percent body area of each color patch in ADOBE PHOTOSHOP (Adobe Systems, San Jose, CA), with head and dorsal regions accounting for 88% and remaining areas 12%. Hence, each ΔS and ΔL is a weighted function of the relative body area for each color patch, producing a measure of whole body conspicuousness that is more appropriate than single patch comparisons (21). Conspicuousness viewed from above was evaluated as the Euclidean distance of color and brightness contrast, $E = \sqrt{\Delta S^2 + \Delta L^2}$, producing vector distance in a perceptual space. Confidence ellipses (95%) were calculated for each species (Fig. 1; *E. pa l*, $n = 24$; *A. apa o no Y*, $n = 11$; *E. biling i*, $n = 19$; *A. apa o Y*, $n = 17$; *E. hahneli*, $n = 10$; and *A. femo ali*, $n = 12$). We used a Kruskal–Wallis nonparametric test for all comparisons among Euclidean distances.

Predator Learning Experiments. Predator learning experiments generally followed methods as described in ref. 3. Although few data exist, birds may be potential poison frog predators (16–18). Thus, in Quito, Ecuador, we conducted a series of learning experiments using ≈ 1 -mo-old domestic chickens (*Gall gall dome ic*) as naïve model predators (35) and wild-caught dendrobatids (toxic species, *E. biling i*, *E. hahneli*, and *E. pa l*; nontoxic species, *A. femo ali*, *A. apa o Y*, and *A. apa o no Y*). Birds were tested individually in a 1-m² dirt-floor test arena.