Contrasting colors of an aposematic poison frog do not affect predation

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Warning signals of aposematic organisms often include patterns that contrast with background coloration, though controversy exists over their importance. Many dendrobatids have contrasting colors, but no work has established whether these are anti-predator components of the warning signal. We used 840 clay frog models to test whether a black spotted pattern on the red dorsum of the poison frog Oophaga pumilio (= Dendrobates pumilio) from Costa Rica enhances the aposematic signal. Model type, patterned or not patterned, did not predict predation. However, we did find evidence that background (i.e., contrast between an aposematic organism and its environment) influenced a predator’s attack decision because models on white paper (higher contrast) were attacked significantly less than models on leaf litter (lower contrast). Our results indicate that the pattern of Costa Rican O. pumilio does not influence predation. Our results also support the hypothesis that novel backgrounds evoke a neophobic reaction and can affect predation rates.

Introduction

Understanding how selection acts on individual traits has been a central question in biology since the concept was first introduced by Darwin. Aposematic organisms provide an excellent system for studying the effects of selective pressures because of their easily recognizable traits (i.e., conspicuous colors, sounds, etc.). Organisms said to be aposematic are those that possess both a secondary defense and warning signal that hasten predator learning of unprofitability (Wallace 1878, Poulton 1890, Ruxton et al. 2004, Mappes et al. 2005). Predator aversion to aposematic organisms is considered either an innate (Smith 1975, Pough 1988) or learned behavior (Lindström et al. 2001, Ruxton et al. 2004). Aposematic signal characteristics are influenced by predation and vice versa (Gamberale-Stille & Guilford 2004), and it has been suggested that
predation pressure maintains aposematic traits (Gamberale-Stille & Guilford 2004, Ruxton & Speed 2006). Puurtinen and Kaitala (2006) suggest that selection for aposematism increases with predation intensity, yet the development of ‘optimal’ warning signals is poorly understood (Speed & Ruxton 2007).

Many aposematic organisms exhibit colors that show strong contrast, usually red or yellow in contrast with black (Cott 1940). Contrast within the aposematic display and its surrounding environment is expected to increase the effectiveness of the warning signal through increased conspicuousness and distinctiveness from palatable prey (Sherratt & Beatty 2003, Endler & Mappes 2004, Merilaïta & Ruxton 2007, Gamberale-Stille 2008). Contrasting elements (e.g., spots and lines) provide edges in aposematic organisms because they contrast with the base coloration, which may facilitate detection by predators through lateral inhibition, which is the physiological mechanism by which organisms detect edges (Forsman & Herrström 2004). Size of the contrasting pattern elements within the warning display is also predicted to affect conspicuousness. In general, larger pattern elements are predicted to enhance a predator’s ability to learn avoidance of the aposematic organism (Hagman & Forsman 2003, Sherratt & Beatty 2003, Lindstedt et al. 2008).

Multiple selective forces acting on the same elements can complicate the study of contrasting pattern elements. The potential for multiple selective forces to act on a particular trait makes identification of the role of any single selective agent difficult (Ojala et al. 2007, Friman et al. 2009, Lindstedt et al. 2009). Although theory generally predicts that contrasting colors lead to increased conspicuousness, some evidence demonstrates both the importance and unimportance of contrasting colors with respect to predation, in both aposematic and non-aposematic species (Aoki et al. 2000, Rowe & Guilford 2000, Forsman & Herrström 2004, Prudic et al. 2007, Aronsson & Gamberale-Stille 2008). In some cases, naïve chicks avoid distasteful prey with black patterns more often than distasteful prey that lack black patterns (Rowe & Guilford 2000, Forsman & Herrström 2004). Black spots in non-aposematic guppies resulted in increased levels of conspicuousness, which was indicated by increased predation by fish and invertebrates (Endler 1978). However, a more recent study on the importance of contrasting colors using naïve chicks found that avoidance was greater for colored prey than prey with a contrasting black pattern (Aronsson & Gamberale-Stille 2008).

Contrasting colors are present in the visual displays of many neotropical, brightly colored dendrobatid frogs, but research has not focused on the role that contrasting colors play in their aposematic signal. Wollenberg et al. (2008) found that four pattern elements did not correlate with a neutrally evolving gene in Dendrobates tinctorius and suggested that the pattern elements were likely under selection. Rudh et al. (2007) examined whether or not spot characteristics (i.e., spot size and percent coverage of spots) in Oophaga pumilio were under selective pressure, and concluded that variation in spot pattern was the product of either sexual selection or differences in predation pressure. Although predation might be one of the main selective forces responsible for contrasting colors in aposematic frogs (Summers et al. 1999, Summers et al. 2003, Reynolds & Fitzpatrick 2007), empirical data to test this hypothesis are lacking.

Dendrobatids (commonly referred to as poison frogs) are a monophyletic group of frogs that occur throughout parts of Central and South America (Grant et al. 2006). Brightly colored dendrobatids are chemically defended by the presence of alkaloids, which are sequestered from dietary sources and stored in dermal granular glands (Neuwirth et al. 1979, Daly et al. 1994, Saporito et al. 2004, 2007a, 2010). Predation upon dendrobatids is not observed frequently, although anecdotal evidence of birds and snakes consuming, or attempting to consume dendrobatids have been described (e.g., Silverstone 1975, Saporito et al. 2007b, Toledo et al. 2007). Experiments with ants and spiders have shown that these invertebrates find certain dendrobatids unpalatable (Myers et al. 1978, Brodie & Tumbarello 1978, Fritz et al. 1981, Myers & Daly 1983, Szelistowski 1985, Master 1998, Gray et al. 2002, Saporito et al. 2007b, 2007c). Saporito et al. (2007b) found that the majority of predators of Oophaga pumilio were birds, which have color vision and can detect the bright col-
oration of this species (Siddiqi et al. 2004).

The strawberry poison frog *Oophaga pumilio* ranges from the Caribbean lowlands of southern Nicaragua through Costa Rica and into the northwestern portions of Panama (Savage 1968, Silverstone 1975, Myers & Daly 1983). Over most of its geographic range, *O. pumilio* has a reddish-orange body with blue-black legs (Savage, 2002, Guyer & Donnelly, 2005), yet in Bocas del Toro, Panama this species exhibits a high degree of polymorphism in color and pattern (see Myers & Daly 1983, Rudh et al. 2007, Saporito et al. 2007c). Siddiqi et al. (2004) examined the ability of birds to detect some of the different colored frogs in Bocas del Toro, but intentionally did not consider the pattern elements. Using clay model replicas of frogs, Saporito et al. (2007b) experimentally demonstrated that the red coloration of *O. pumilio* in northeastern Costa Rica functions as an aposematic signal to natural predators; however, this experiment also did not examine the importance of pattern to the aposematic signal. Herein, we test whether the presence or absence of the spot pattern of *O. pumilio* in northeastern Costa Rica affects predation. Specifically, we examine whether or not the presence of spotting pattern results in decreased levels of predation and if this spot pattern might result in protection through crypsis.

**Methods**

**Study location and general methods**

This study was completed at the La Selva Biological Research Station in Costa Rica from 30 May 2008 through 21 June 2008. Spotted and non-spotted individuals of *O. pumilio* are found throughout La Selva and the majority of the population is spotted (74.7%, n = 59/79), though areas with equal proportions of spotted and non-spotted frogs also exist (M. A. Donnelly & R. A. Saporito unpubl. data). Therefore, we can safely assume predators regularly encounter both morphotypes.

To examine the potential effect of the presence of spots on predators we used polymer clay models of *O. pumilio* constructed from nontoxic, pre-colored modeling clay (Sculpey-III®) (Fig. 1). Polymer clay models are widely used in studies of predation in vertebrates and many previous studies of aposematism, as the use of actual vertebrate animals would pose ethical dilemmas. Systems that have successfully used polymer clay models include snakes (Brodie 1993, Niskanen & Mappes 2005), salamanders (Kuchta 2005), and poison frogs (Saporito et al. 2007b, Noonan and Comeault 2009). Clay models have also been widely used to study fruit color preference by tropical birds (Alves-Costa & Lopes 2001). The soft clay models are especially beneficial as they record predation attempts through bite imprints left on the clay surface that allows the identification of potential predators. Additionally, clay models accurately represent real prey to natural predators, which permits inference about the evolution of aposematic characteristics in a real predator/prey system.

To ensure the color of frog models matched the color of *O. pumilio*, we used color data collected from 101 *O. pumilio* from La Selva that
were obtained by matching frog dorsal color to a color chart (Online Auction Color Chart, www.onlineauctioncolorchart.com; M. A. Donnelly & R. A. Saporito unpubl. data). We then created mixtures of different clay colors to best match the most common body color of *O. pumilio* (red no. 649), and used a spectrophotometer (PP SYSTEMS Unispec-SC) to match the wavelengths of the color chart with our models. Spectrophotometric measurements were made under standard light conditions. Spot diameter, the number of spots, and spatial arrangement of the black spots were standardized across all models. The spatial arrangement of spots matched that of the real frogs. We scored spot characteristics from 30 images of *O. pumilio* from the La Selva population (Donnelly and Saporito, unpublished data). Average spot diameter was 0.50 mm (SD = 0.02) and the average number of spots was 90 (SD = 6.14).

We constructed the clay models using a rubber mould. We measured 10 preserved *O. pumilio* housed in the herpetology collection at Florida International University to create the frog model used to make the rubber mould. Legs made of blue clay were attached by hand to the red body of the frog model created in the mould. Black eyes and black dorsal spots were drawn on the frogs with a permanent marker that did not reflect ultraviolet (UV) light, because *O. pumilio* do not reflect UV light (Summers et al. 2003).

Twenty models (10 spotted and 10 non-spotted) were placed at 5-m intervals along 100-m transects. The model type (spot/no spot) at each placement point was randomized with a coin toss, but no more than two of any model type were placed consecutively along a transect to evenly distribute model types. Transects were placed at least 100 m apart. A total of 42 transects were used in this study with 840 frog models (420 spotted and 420 non-spotted). We placed 14 transects in old-growth, secondary, and agro-forestry habitats to ensure our study covered a range of diverse habitats present at La Selva. Half of the transects contained models placed on white ‘Rite in the Rain’ paper (9.5 × 10.5 cm) to remove any cryptic effects spots might have (Brodie 1993, Saporito et al. 2007b); the other transects had models placed on the leaf litter (a brown background).

**Predation assessment**

Models were placed in the forest for 48 hours before being collected and scored as in previous studies (Brodie 1993, Saporito et al. 2007b). The number of attacks per 48 hours on models was recorded and used to estimate ‘predation rate’. In an attempt to avoid counting multiple attacks by a predator as different predation attempts, all models with more than one attack mark were counted as a single predation attempt. All attacked models were photographed, tagged, and stored. Predation attempts were classified by predator type. Marks on models were classified into five categories: bird, ‘potential bird’, mammal, arthropod, and unknown. Birds left U- or V-shaped imprints on the model (Fig. 2). Models with marks classified as ‘potential bird’ contained markings that resembled those made by birds, but did not have clear U- or V-shaped imprints from beaks. Mammalian predation was distinct because the spaces between teeth leave distinct ridges and marks. Arthropods, particularly ants, made small, paired linear marks on the model’s surface with their mandibles. Lastly, marks made on the model surface that were inconsistent with any of the other four categories were placed in an ‘unknown’ category.

**Statistical analyses**

Binary logistic regression was used to determine if presence of spots, background type, and/or habitat were a significant predictor of predation rate, and to examine potential interactions among the three variables (which were all categorical). Logistic regression has some advantages over Chi-square methods, which have been used more extensively in previous studies (see Brodie 1993, Noonan & Comeault 2009). First, empirically testing for an interaction between factors (predictors) and interpretation of results are straightforward. A second benefit is that both continuous and categorical explanatory variables can be included in the model. Third, it is possible to test one-sided directional hypotheses. Finally, in addition to producing tests of significance, logistic regression includes estimates of effects.
tic regression is in the form of the odds ratio, a number that signifies the amount by which the odds of predation change for every one-unit change in the predictor value (or for a change from one category to the another in the case of a categorical predictor). The odds ratio works multiplicatively; for example, an odd ratio of 1.5 would mean that the odds of predation increase by 1.5 (or 50%) for every one unit change in the predictor value.

All possible models (from each variable on its own, to the full model with all three variables and all three two-way interactions) were examined. The final model did not include interactions, though, because none were found to be significant. The effect of habitat type was not of primary interest, but we include it to account for its effects.

Data were analyzed in three ways that differed in the conservativeness of what we defined as a positive predation event, though the main results did not change. We first considered models attacked only by birds as the most conservative estimate of predation, and because birds possess color vision that enables them to see the whole aposematic signal. Secondly, we expanded the definition of predation to include models attacked by birds, those potentially attacked by birds, and missing models. Missing models were included in the second analysis because occasionally we found a missing model several meters from its original location with bird beak imprints. Therefore, the second analysis represents a less conservative estimate of avian predation because we include inferred predation events from these cases. Brodie (1993) stated that consecutive attacks along the same transect could be the result of a single predator. Therefore, in a third analysis we removed consecutive attacks to limit any influence repeated

Fig. 2. Examples of clay frog models attacked by a (A) bird, (B) potential bird, (C) mammal, and (D) arthropod.
attacks along a transect by the same predator might have on our results. All statistical analyses were done using Minitab ver. 15.

**Results**

In all three analyses (with responses connoting conservative bird predation, “less conservative” bird predation, and without repeated attacks), there were no significant interactions (interaction term \( p > 0.25 \) in all cases). Therefore, in each case, we will restrict our discussion to the effect of the three primary predictors.

A total of 104 (12.4%) clay models of *O. pumilio* had marks on the surface (Fig. 3). The percentage of clay models attacked is comparable to that in other studies of predation in aposematic organisms (see Brodie 1993, Saporito et al. 2007b, Noonan & Comeault 2009). In the conservative estimate of avian predation, 21 non-spotted models were attacked and 29 spotted models were attacked (Fig. 3). Thirty models attacked by birds were on leaf litter and 20 models were attacked on paper. Model type (spotted or non-spotted) did not predict predation (\( p = 0.245 \), odds ratio = 1.41, CI95 = 0.79–2.52). When consecutive predation attempts were removed, the result did not change (data not shown). The background type (paper or leaf litter) did not predict predation (\( p = 0.147 \), odds ratio = 1.54, CI95 = 0.86–2.76).

Under the expanded definition of avian predation, which included bird attacks, potential bird attacks, and missing models, 37 spotted models and 40 non-spotted models were attacked (Fig. 3). When the attacked models are divided by background type, 47 models were attacked on leaf litter and 30 models were attacked on white paper. Under the relaxed predation definition, model type did not predict predation (\( p = 0.634 \), odds ratio = 1.12, CI95 = 0.704–1.79). Background type, though, was a significant predictor of predation (\( p = 0.034 \)). The odds of being attacked on a white background were 43% lower (CI95 = 7%–65%) than on forest leaf litter (brown) backgrounds.

**Discussion**

We found that spot pattern of *Oophaga pumilio* did not influence predation rate, which suggests that spots do not enhance or reduce the effectiveness of the aposematic signal in the La Selva population in northeastern Costa Rica. Our results support the findings of Aronsson and Gamberale-Stille (2008), who found pattern elements to be less important than coloration in warning displays. The spot pattern associated with the population of *O. pumilio* examined in the current study appears to carry no significant cost or benefit to the aposematic display, and suggests that pattern may be a neutral trait with respect to predation. However, the majority of *O. pumilio* at La Selva are spotted, which suggests that pattern may be important to aspects other than aposematism, such as sexual selection (see Reynolds and Fitzpatrick 2007, Maan and Cummings 2008).

One potential explanation for why we observed no difference in predation on spotted and non-spotted individuals was the small size of spots, which may have resulted from past selection pressures. Larger pattern elements are predicted to increase visibility (Lindstedt et al. 2008) and avoidance learning by predators of aposematic prey (Lindström et al. 2001, Hagman...
& Forsman 2003, Sherratt & Beatty 2003, Lindstedt et al. 2008). In Bocas del Toro, Panama, spot size is more variable than it is in La Selva frogs, and individuals often have spots that are larger in size than those of frogs in more inland areas of the species range. A potential explanation for why frogs at La Selva and in most places outside of the Bocas del Toro region have small spots, is that individuals with larger spots may be prone to greater predation. Data suggest that predation pressure in Bocas del Toro is smaller than in Costa Rica, perhaps permitting more conspicuous, larger, spot sizes that would make individuals in Costa Rica more conspicuous to predators unaffected by defenses (Hegna 2009).

The coefficient of variance ($\sigma/\mu$) for the number of spots ($CV = 6.8\%$) is also 1.7 times greater than for spot size ($CV = 4\%$) in the 30 individuals collected in La Selva and analyzed prior to the experiment that supports our observation that spot size is potentially under greater selection for decreased size at La Selva. The presence of small spots in this population of *O. pumilio* in Costa Rica suggests a possible trade-off between being visible enough to ward off most potential predators and being cryptic enough to avoid the few predators that view *O. pumilio* as a food source. A potential trade-off by *O. pumilio* could be an effort to balance detection risk with maintaining a signal that remains effective at educating predators to avoid the chemically defended frogs and warrants further study (Endler & Mappes 2004, Lindstedt et al. 2008).

Although we originally hypothesized the presence of spots would increase the visibility of frogs to predators, the general spot pattern (small dots spread over the dorsum) also suggested the possibility that spots were involved in crypsis by disrupting the outline of the frog or background matching. In aposematic displays, pattern elements can help balance the conspicuousness of a warning signal against background coloration by partially distorting the outline of an organism, which may provide protection from the few predators that target aposematic prey (Cuthill et al. 2005, Schaefer & Stobbe 2006, Stevens et al. 2006). Therefore, contrasting colors might serve to counteract a background that makes the aposematic organism stand out (i.e., more conspicuous), such that it is more prone to predators unaffected by the defense (Gamberale-Stille 2001, Cuthill et al. 2005, Stevens & Cuthill 2006). In addition, distortion of the body outline as a result of contrasting colors could allow the organism to be cryptic from a distance and conspicuous only when in close proximity to the predator (Tullberg et al. 2005). We investigated whether or not spots on *O. pumilio* increased crypsis by manipulating background type (paper or leaf litter). Our results show that attack rates on spotted and non-spotted frogs did not differ for models on white paper or models on leaf litter. Therefore, the presence of spots on our models appears to not produce a more cryptic display as compared with frogs without spots. Had spots provided a benefit through increased crypsis, we would have predicted fewer attacks on spotted models placed on leaf litter.

In addition to internal contrasts between the pattern elements and the base coloration, contrast between the aposematic organism and background coloration is also important to consider. In our experiment, we used white paper to make both types of models equally visible in case the presence of spots had a cryptic effect as discussed in the previous paragraph. Background type (leaf litter or white paper) was a significant predictor of predation under a less conservative estimate of avian predation (see Results). It is possible that white paper induced a neophobic reaction in predators. Lindstedt et al. (2008) also found a similar trend in experiments where a green background resulted in increased conspicuousness of aposematic moth larvae that subsequently decreased attacks by naïve chicks. Many predators exhibit some degree of neophobia and combined with natural dietary conservatism (short and long term aversion to novel food items respectively), it may be at least one explanation for how conspicuous aposematic prey initially evolved (Thomas et al. 2003, 2004, Marples et al. 2005). Saporito et al. (2007b) also observed decreased attacks for both red and brown models on white paper, but the trend was marginally not statistically significant. While our experiment was not intended to test neophobia as a force that could facilitate the evolution of aposematism, our results suggest that predators in La Selva avoided models presented in a novel context (i.e., an artificial substrate). Therefore,
our results appear to support the hypothesis that novel backgrounds can potentially facilitate the initial evolution of aposematism (Gamberale-Stille 2001, Cuthill et al. 2005, Schaefer & Stobbe 2006). The influence of novel backgrounds on predation remains a heavily debated issue, though, as other studies exist showing no influence on predation rate when novel backgrounds are used (see Lindström et al. 1999, Niskanen and Mappes 2005). Our results make an important contribution to the understanding of whether novel contexts can aid in the evolution of aposematic traits, especially given that this is a natural, rather than laboratory experiment. Additionally, these results raise the question of whether contrast within the warning signal, or contrast between the organism and its environment is more important to warning signal effectiveness (Gamberale-Stille 2001), or whether contrasting backgrounds improve signal efficiency in general. It is likely that whether internal or external contrasts are more important to aposematic signals is context dependent on size, shape, placement or other pattern characteristics and additional experiments in this system could provide insights to the process.

Overall, we found that contrasting colors in models of prey organisms did not affect attack rates by predators, which supports the findings of Aronsson and Gamberale-Stille (2008) who concluded pattern was less important to aposematic signals. Our experiment is the first to our knowledge to demonstrate that contrasting colors are not important to the signal of an aposematic organism using natural predators. Our results also indicate that the small spots present on the mainland frogs in Costa Rica appear to be a ‘neutral’ trait with no predation cost. Larger spots observed on *O. pumilio* in the Bocas del Toro Archipelago of Panama might make the frogs more prone to predators unaffected by the defenses, though this remains untested at the moment. Müllerian mimicry theory predicts that variation in warning signals should be selected against (Joron & Mallet 1998), yet our data indicate that some aspects of warning signals are potentially able to vary without cost. Additionally, we show evidence that background coloration can potentially be a significant influence on whether a predator chooses to attack. The patterns of dendrobatids are diverse, which suggests different factors can influence pattern elements in different species (e.g. sexual selection, predation, thermoregulation) and experiments with models allow for future tests concerning aposematism in brightly colored frogs. Whether or not contrasting colors enhance the display of aposematic organisms is undoubtedly complex and it is unlikely that there is a single unifying theme, but rather it appears that the importance of contrasting colors varies across species and possibly within species under different contexts.

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