

Not everything is black and white: Color and behavioral variation reveal a continuum between cryptic and aposematic strategies in a polymorphic poison frog

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Abstract

Aposematism and crypsis are often viewed as two extremes of a continuum of visual conspicuousness to predators. Theory predicts that behavioral and coloration conspicuousness should vary in tandem along the conspicuousness spectrum for antipredator strategies to be effective. Here we used visual modeling of contrast and behavioral observations to examine the conspicuousness of four populations of the granular poison frog, *Oophaga granulifera*, which exhibits almost continuous variation in dorsal color. The patterns of geographic variation in color, visual contrast and behavior support a gradient of overall conspicuousness along the distribution of *O. granulifera*. Red and green populations, at the extremes of the color distribution, differ in all elements of color, contrast and behavior, strongly reflecting aposematic and cryptic strategies. However, there is no smooth cline in any elements of behavior or coloration between the two extremes. Instead populations of intermediate colors attain intermediate conspicuousness by displaying different combinations of aposematic and cryptic traits. We argue that coloration divergence among populations may be linked to the evolution of a gradient of strategies to balance the costs of detection by predators and the benefits of learned aversion.

The origin and prevalence of intraspecific variation in animal coloration is a complex and fundamental question in biology, because color may simultaneously be subject to multiple evolutionary forces. Most evidence indicates that, in species with distinct polymorphism or polytypism, color is under selective pressure (e.g. Andrés et al. 2000, Rosenblum 2006, Brown et al. 2010), though a few examples exist of intraspecific color variation maintained solely by genetic drift (e.g. Hoffman et al. 2006). Thus, potential mates (sexual selection) and predators (natural selection) are often invoked as the selective agents producing color divergence within a species (reviewed in Gray and McKinnon 2007). A vast body of literature suggests that variation in female preferences for male traits can cause fast color divergence (e.g. McKinnon and Rundle 2002, Maan et al. 2006, Reynolds and Fitzpatrick 2007), for instance if conspicuousness of differently colored males varies across environments (Boughman 2001). However, differences in conspicuousness may also result from predation, and for many animals the role of visually oriented predators driving the evolution of color patterns is ubiquitous (Ruxton et al. 2004).

Animal coloration may prevent predator attacks in multiple ways. For example, color can impede detection in cryptic prey or act in synergy with toxicity to discourage predation attempts as in aposematic animals. However, these are just the two extremes of a continuum of possible antipredator strategies. Various theoretical and empirical studies have analyzed the conditions in which intermediate levels of conspicuousness may arise (e.g. Wüster et al. 2004, Darst et al. 2006, Ruxton et al. 2009, Speed et al. 2010). For instance, intermediate levels of conspicuousness may be favored by natural selection in chemically defended prey if

the detection costs of high conspicuousness outweigh the benefits of aversion learning (Ruxton et al. 2009), or if some predators can manage toxic prey rather than avoid them at all encounters (Endler and Mappes 2004). Similarly, once aposematism is established, different combinations of toxicity and conspicuousness levels may evolve yielding similar predator deterrence (Darst et al. 2006). Additionally, effects of predators or sexual preferences of potential mates may also drive color variation in species that occupy heterogeneous habitats, where prey or mates are encountered under different light conditions (Galeotti et al. 2003, Dugas and Franssen 2011) or on different backgrounds (Rosenblum 2006), since both light and background, affect conspicuousness.

For an antipredator strategy to be successful coloration is expected to evolve in tandem with behavior (Mochida 2009, Speed et al. 2009, Pröhl and Ostrowski 2011). The rate of various behavioral elements, such as movement, foraging and sexual displays influence conspicuousness of prey, as well as conspicuousness influences the escape behavior of prey (e.g. Galatowitsch and Mumme 2004, Pröhl and Ostrowski 2011, Ozel and Stynoski 2011). Therefore, behavioral traits that increase exposure should be favored in aposematic animals (Speed et al. 2009). However, only a few studies have addressed the correlation between behavioral and coloration conspicuousness, and most of them are based on comparisons across species. In general, these studies support the notion that aposematic species have higher movement and foraging activity, whereas resource exploitation by cryptic animals is restricted to habitats where background matching is attained, and thus these species are usually less mobile (Stamp and Wilkens 1993, Bernays and Singer 2002, Merilaita and Tullberg 2005). Evidence from chemically defended species with variable color patterns or antipredator strategies sustains this idea. Caterpillars with ontogenetic color change have more conspicuous foraging behaviors during the last instar, when they are brightly colored and aposematic (Grant 2007). Similarly, locusts that transit from a solitary and cryptic phase

to a gregarious and aposematic phase exhibit higher movement and lower latency to feed when gregarious (Despland and Simpson 2005). Furthermore, Mochida (2009) showed that aposematic newts that had a large brightly colored ventral patch also had higher tendency to perform the Unken reflex -a behavioral enhancer of the aposematic signal-than newts with smaller patches. Although these studies reveal clear behavioral differentiation between contrasting antipredator strategies and a correlation between behavior and color pattern within the aposematic strategy, studies including individuals of intermediate conspicuousness are needed to test if behavior and coloration are correlated across the whole spectrum between crypsis to aposematism. Here, we examine the correlation between behavioral and color conspicuousness across four populations of a poison frog that exhibits almost continuous variation in dorsal color pattern, including multiple phenotypes of intermediate conspicuousness.

In Neotropical poison frogs, at least three independent origins of bright coloration have produced dramatic color variation among species (Santos and Canatella 2003), whereas subsequent losses of these bright colors have caused some of the most spectacular examples of intraspecific color polymorphism among vertebrates (Wang and Shaffer 2008, Wang 2011). Indeed, color morphs of the strawberry poison frog, *Oophaga pumilio*, span the range from blue to red colorations, including green, yellow and orange morphs (Summers et al. 2003). Current evidence supports a role for sexual selection driving rapid color divergence in this species, because females of *O. pumilio* usually prefer males from their own color morph (Summers et al. 1999, Reynolds and Fitzpatrick 2007, Maan and Cummings 2008). However, variation in female preferences may in turn be driven by natural selection if the strength of the aposematic signal is reduced in hybrids, which exhibit intermediate coloration (Summers et al. 2004), and if these intermediate phenotypes are attacked by predators more often. Additionally, evidence from other species, *Dendrobates tinctorius* and *Ranitomeya imitator*,

suggests that selection by predators can maintain color divergence when morphs are allopatric, because novel and conspicuous phenotypes would suffer higher predation (Noonan and Comeault 2009, Chouteau and Angers 2011).

Color divergence in some poison frog species has resulted in differences in conspicuousness to putative predators, including birds (Sidiqqi et al. 2004, Pröhl and Ostrowski 2011, Wang 2011), snakes and crabs (Maan and Cummings 2012). Furthermore, contrasting color morphs of *O. pumilio* exhibit marked behavioral differences in accordance to predictions of aposematic and cryptic strategies (Pröhl and Ostrowski 2011, Rudh et al. 2011). The granular poison frog in Costa Rica, *Oophaga granulifera*, also includes populations of contrasting red frogs in the South and green frogs in the North, but at intermediate latitudes several populations of intermediate colors have been found (Savage 2002, Wang 2011, Brusa et al. 2012). Here, we analyze the behavior and visual contrasts to putative predatory birds under field conditions, of two populations of extreme and two populations of intermediate color morphs of *O. granulifera*. If predators have influenced color divergence in this species, then it is expected that behavioral elements of antipredator strategies vary in tandem with coloration across multiple color morphs.

METHODS

Frog populations

O. granulifera is distributed along the southwestern lowlands of Costa Rica and adjacent northwestern Panamá (Savage 2002). The frogs have a red dorsal coloration in most of the species range, but in the northern part of its distribution populations of dorsally yellow and dorsally green frogs have been reported (Savage 2002, Wang 2011). Recently, a number of intermediate populations with intermediate coloration have been discovered between the

green and red localities (Brusa et al.2012). We selected four populations of *O. granulifera* (Fig. 1), aiming to include most of the color variation observed in the species. In San Rafael most frogs exhibit green dorsal coloration, while in Portalón dorsal colors include green, yellow, bronze and orange. Frogs are orange or red in Matapalo and red in Barú. All populations exhibit variation in ventral color pattern; the ventral surface can be turquoise, of the same color as the dorsum, or have distinct areas of turquoise and the dorsal coloration. The study populations (hereafter green, intermediate-green, intermediate-red and red) ranged from occupying very disturbed habitats surrounded by villages and farmland in the intermediate-green and intermediate-red populations to a rather mature forest in the green and red populations. Frogs were frequently found near fast-moving streams, often on steep slopes and rock boulders, however the red frogs were also abundant at an abandoned banana plantation under natural restoration.

Coloration measurements and visual modeling

We obtained reflectance spectra $R_f(\lambda)$ from 300-800 nm of dorsal and ventral frog skin using an Ocean Optics bifurcal optic fiber R-200-2-UV/VIS connected to an Ocean Optics HR2000 spectrometer. The probe was positioned perpendicular to the surface of the frogs and at a distance of 2 mm. We used a deuterium-tungsten DT-Mini-2-GS as a light source and calibrated with a WS-1-SS white standard every eight measurements to account for lamp drift. All color measurements were taken in a dark room to exclude ambient light. The reflectance spectra of each frog were averaged over eight dorsal measurements (two of the head and six of the dorsum) and eight ventral measurements (two of the throat and six of the belly). All focal males (see “Behavioral observations”) were measured, as well as other frogs found during the study period. Also, samples of all substrates where focal males called during the observations were collected and reflectance spectra for the substrates $R_s(\lambda)$ were obtained

as above with five measurements per sample. A single male could use one to seven different substrates during the observations, thus substrates were grouped in six categories according to their reflectance spectra: 1) trunks, including all kinds of tree bark (e.g. tree trunks, buttresses, fallen logs); 2) rocks, which were typically covered with moss; 3) green leaves, mostly *Dieffenbachia*; 4) leaf-litter; 5) banana plants and 6) bare soil. To define population specific luminance conditions we measured irradiance $I(\lambda)$ -the spectrum of light that enters the eye - using an Ocean Optics cosine adaptor head (CC-3UV) attached to an optic fiber QP-400-2-UV-BX and connected to the HR2000 spectrometer. At each locality, irradiance was measured at 25 frog calling sites and over five days (five sites per day), except for the intermediate-red population where 10 spectra were recorded over two days. We recorded irradiance spectra at 6:00 and 9:00 in the morning, but these two measures were averaged since they produced virtually identical results.

To describe the color of each population we calculated the overall brightness, hue and chroma of the dorsal and ventral skin of frogs following Endler (1990), and assuming frogs are seen under the same conditions. The brightness indicates the total reflectance flux, while the hue is given by the dominant wavelength of reflected light, and the chroma describes the distribution of $R_f(\lambda)$ across the spectra wavelengths (i.e. the saturation of the color). These are viewer-independent measures of color calculated over 400-700nm, that have some general properties of all visual systems.

Conspicuousness to potential predators was evaluated with visual modeling, developed by Vorobyev et al. (1998) and modified by Stuart-Fox et al. (2003) and Siddiqiet al. (2004). The color and luminance contrasts between the skin (dorsal or ventral) of the frogs and each type of substrate were calculated for the visual system of a model predatory bird (*Parus caeruleus*). Thus the inputs of the models were $R_f(\lambda)$, $R_s(\lambda)$, $I(\lambda)$ and $S_i(\lambda)$ for all λ between 300-800nm, and where S_i is the sensitivity of the taxon specific cone type i . The

integration interval of the models was of 1nm. The model also accounted for the noise to signal ratio of the different cone types. Detailed equations for this model are presented in Appendix 1 of Pröhl and Ostrowski (2011).

We compared color elements (brightness, hue, and chroma) across frog populations using multivariate analyses of variance (MANOVAs), since these elements are strongly correlated. We then provided univariate analyses of variance (ANOVAs) to aid the interpretation of the overall multivariate effects. To compare light environments of the four populations we conducted an ANOVA using $\sum_{\lambda} I(\lambda)$ as the response variable. We assessed inter-population differences in color and brightness contrast for the dorsal and ventral skin and for bird and frog vision using ANOVAs. Color and luminance contrasts were compared at the four substrate types most commonly used (trunks, rocks, leaves and leaf-litter) and the sequential Bonferroni (Rice 1989) correction was applied to control the type-I error rate for each substrate ($k=4$). Banana plants were excluded because they were only used by red frogs. The bare soil was excluded because only six frogs ever called from it and only from the intermediate-red and red populations. For each frog population, contrasts were calculated using the substrate and irradiance data of their own locality since modeling frog contrast with the substrates and irradiance of other localities does not qualitatively affect our results (Supporting Information S1).

Behavioral observations

To quantify behavioral elements that correlate with antipredator strategies we conducted behavioral observations of focal males at each locality. All behavioral data was recorded by a single observer, during the wet season (June-November) of 2011. Due to differences in accessibility to the populations sample sizes varied among them: 10 green, 18 intermediate-green, 6 intermediate red and 12 red males were observed. Each frog was observed

continuously for 15 min on 10 different days. Males of *O. granulifera* are active mostly during the early morning (5:30 to 9:00) and again in the late afternoon (Bolaños 1990, Savage 2002). Thus our observations, conducted between 5:00 and 10:00 in the morning, were timed to capture most of the frogs' activity. Males are strongly territorial (Savage 2002), some of them were observed on the exact same spot every morning over several weeks or even months (Willink and Bolaños personal obs.). This strong territoriality, combined with the high variation in leg and ventral color pattern and unpigmented scars allowed us to readily identify individual males without toe clipping.

Six to ten observations were conducted every morning in a randomized order, at a distance of at least 2 m from the frog. We recorded: the time (s) the frog spent calling (calling activity), the number of feeding events, the number of minutes in which the frog moved over a distance of at least 5 cm, and the number of minutes in which the frog remained inactive (i.e. not moving, calling or foraging). We only analyzed calling activity that occurred when there were no females interacting with focal males, to account for differences in male motivation. For every observation in which a male called we also recorded the type of substrate(s) where the frog called from, and estimated exposure of the male while calling. To estimate exposure, we placed a real size clay frog model at the exact place(s) where the frog had been calling and took four lateral pictures forming 90° angles around the clay frog and one picture from right above the frog, with a Cannon Lumix DMC-LZ8 camera. To take these pictures the camera was placed at a distance of 1 m from the clay frog, and for the lateral pictures at the same height as the frog. The pictures were downloaded into a computer and we estimated the proportion of the frog's body visible in the pictures (0, 0.25, 0.5, 0.75, or 1). A final exposure value was calculated for each frog as the average exposure from the pictures weighted by the time the frog spent calling from each position.

Behavioral data were analyzed with generalized linear models (GLM). For the calling activity, inactivity and movement data, a binomial error distribution was assumed. The distribution is binomial because every second (calling activity) or minute (movement and inactivity) the male frogs may or may not have engaged in each one of the behaviors. Thus, the response variables of the GLMs were the proportion of time in which the focal males performed each behavior. We report association tests between the frog population and the probability of calling, moving or remaining inactive. The analysis on foraging data was performed assuming a Poisson distribution of errors, because the data are counts of feeding events. We used quasidistributions to account for overdispersed data. The effects of the frog population on the exposure of calling males were evaluated with ANOVAs.

Escape behavior

We recorded the escape behavior of 56 calling males, 8 green, 21 intermediate-green, 9 intermediate-red and 18 red. To elicit an escape response we used a bird model (35.0 cm of wingspan, 18.5 cm of body length and 19 cm of tail length) made of dull green cloth and chicken feathers. The model was attached to a 2 m pole by two strings also attached to the center of each wing. A camouflaged observer moved the pole horizontally towards a calling frog from a starting distance of *ca.* 2 m and at a constant speed. The flight initiation distance, defined as the distance between the frog and the model when the frog hopped away, was recorded as a measurement of frog propensity to escape. We used a Kruskal-Wallis test to assess the effect of the population on the propensity to escape of male frogs.

RESULTS

Coloration and irradiance

We obtained reflectance spectra of 99 frogs, 16 green, 34 intermediate-green, 11 intermediate-red and 38 red. The coloration features of *O. granulifera* varied significantly across populations (dorsal and ventral MANOVA $p < 0.0001$, Table 1). The red frogs had the brightest dorsal skin while the intermediate-green and green frogs were darkest and the intermediate-red had intermediate brightness (Table 2, Fig. 2A). In terms of hue and chroma, the dorsal color of *O. granulifera* seemed to follow a latitudinal gradient, with hue decreasing and chroma increasing from North to South (Table 2, Fig. 2B). However there was no such pattern in the ventral coloration of frogs. Despite the higher brightness of red frogs over intermediate-green and green frogs (Table 2, Fig. 2C), frogs from all populations had similar ventral hue and chroma, except for a small but significant difference in the chroma of intermediate-green and red frogs (Table 1, 2, Fig. 2D). Our study sites varied in light conditions ($F_{3,81} = 8.96$, $p < 0.001$), with the intermediate-green population having darker settings than the red and green populations (both Tukey HSD $p < 0.001$, Fig. 3), and marginally darker setting than the intermediate-red population (Tukey HSD $p = 0.076$, Fig. 3).

Color and brightness contrasts

We evaluated how avian predators perceive the color and brightness contrasts of frogs on their natural backgrounds. A clear pattern of dorsal contrasts emerged across substrates (all ANOVAs $p < 0.001$, see full ANOVA and Tukey HSD tables in Supporting Information S2). Green frogs were less visible than red frogs in terms of both color (Fig. 4A) and brightness (Fig. 4C). The intermediate populations had high color contrasts, similar to the red population, in trunks, rocks and leaves (Fig. 4A), where frogs spent in average 51-70% of

their calling time. However, these intermediate populations tended to have lower brightness contrasts than the red frogs, and intermediate-green frogs were as difficult to detect as the green frogs or even less visible (Fig. 4C). The pattern among ventral brightness contrasts was similar, with red frogs having higher contrast than green and intermediate-green frogs in all substrates, and higher contrast than intermediate-red frogs in two substrates (Fig. 4D). There were statistically significant differences in the ventral color contrasts (all ANOVAs $p < 0.029$), but whether a population had relatively high or low contrast strongly depended on the substrate where they were seen (Fig. 4B).

Frog behaviors

All 46 focal males called at least once during the study period and calling activity significantly depended upon the frog population ($\chi^2 = 10.46$, $df = 3$, $p = 0.015$, Fig. 5A). Red frogs called about 15% of the time, while green frogs spent only 6% of the time calling (Table 3). The two intermediate populations showed high estimates of calling activity, similar to the red frogs (Fig. 5A, Table 3). The foraging rate and the time of inactivity were also dependent upon the population (foraging rate $\chi^2 = 15.10$, $df = 3$, $p = 0.002$, Fig. 5B, inactivity $\chi^2 = 14.24$, $df = 3$, $p = 0.002$, Fig. 5C). Green frogs had lower foraging rates than the frogs from the other three populations and spent more time inactive than the other frogs (Table 3). The movement rate showed a marginally non-significant but similar trend ($\chi^2 = 6.29$, $df = 3$, $p = 0.098$, Fig. 5D), since green frogs had a lower movement rate (Table 3). The red and intermediate-red frogs were more visually exposed while calling than the green frogs ($F_{3,42} = 7.27$, $p < 0.001$, both Tukey HSD $p < 0.005$, Fig. 5E), and marginally more exposed than the intermediate-green frogs (Tukey HSD: red vs. intermediate-green $p = 0.095$, intermediate-red vs. intermediate-green $p = 0.064$, Fig. 5E). The flight initiation distance significantly differed

among populations ($\chi^2 = 9.33$, $df = 3$, $p = 0.025$). The red frogs were more prone to escape from an approaching bird model than the frogs from the other populations (Fig. 5F).

DISCUSSION

Altogether, our results show clear coloration and behavioral differentiation between red and green populations. The intermediate-red frogs resembled the red frogs in most measures of conspicuousness, except in their propensity to escape and some brightness contrasts. On the other hand, the intermediate-green frogs exhibited high color contrasts, movement and foraging rates, displayed intermediate calling activity, and were similar to the green frogs in terms of brightness contrast, calling exposure and propensity to escape. Recently, it has been proposed that intraspecific divergence in color can be linked to distinct antipredator strategies by means of combinations of coloration with sets of behavior that both vary in conspicuousness (Pröhl and Ostrowski 2011). Moreover, in poison frogs the evolution of cryptic phenotypes from aposematic ancestors appears to be common (Wang and Shaffer 2008, Wang 2011, Pröhl and Ostrowski 2011, Rudh et al. 2011). Our results support this general finding and highlight the occurrence of intermediate strategies, that are not purely cryptic or aposematic.

Geographic variation in color and visual contrasts

The dorsal coloration of *O. granulifera* ranges from red to green hues (Wang 2011, Brusa et al. 2012). We found that not only the hue but also the brightness and saturation of color vary with latitude. Such variation is expected to entail reduced conspicuousness from South to North, as shifts to greener hues, lower saturation and lower brightness are predictors of low contrasts in forest environments (Endler 1993). Indeed, for a potential avian predator these

geographic patterns in coloration result in low color contrast of the green frogs and low brightness contrasts of green and intermediate-green frogs against natural backgrounds. Notably, the ventral hue and chroma were similar among populations, except when a large portion of the ventral surface exhibited the dorsal coloration (Fig. 2D, intermediate-green outliers). Consequently, differences in ventral color contrast among populations were always small and determined by the background type. Since birds will normally perceive the dorsal surface of frogs, dorsal but not ventral variation in color pattern is expected if divergence in antipredator strategies drives color variation. To our knowledge, the role of selection on the evolution of ventral coloration has been tested in only one species of poison frogs, where ventral color patterns appear to have evolved neutrally (Wollenberg et al. 2008). Together the pattern of ventral and dorsal contrast variation point to a strong influence of avian predator selection for phenotypic diversity in granular poison frogs.

Antipredator strategies of variously colored frogs

Behavior and visual contrasts reflected strong divergence in aposematic and cryptic strategies between red and green color morphs of *O. granulifera*. The red more conspicuous frogs also engaged in bolder behaviors. Red frogs had higher movement and foraging rates than green frogs and were less concealed while calling. These results are consistent with those of Rudh et al. (2011) and Pröhl and Ostrowski (2011) who respectively compared the exposure while calling and behavior of red and green phenotypes of *O. pumilio*. Pröhl and Ostrowski (2011) also found that green cryptic males preferred to call from substrates where conspicuousness to predators was lowest, but there were no differences in calling activity between color morphs. Unlike Pröhl and Ostrowski (2011), who ignored the social context (absence and presence of females) while observing calling activity, we analyzed calling activity under a single social context and found that when advertising their presence, red frogs had much

higher calling activity than green frogs. The intermediate populations did not display intermediate values in most traits that are assumed to contribute to conspicuousness to potential predators. Instead they reflected the aposematic extreme in some traits and the cryptic extreme in others. However, the combinations of traits of these populations suggest that they are not equally conspicuous, but they both are less conspicuous than the red frogs and more conspicuous than the green frogs.

Fitness of intermediate morphs

There may be different combinations of aposematic and cryptic traits that combined result in intermediate conspicuousness. For instance in lekking birds low brightness contrast with high chromatic contrast and vice versa make a compromise between conspicuousness to conspecific females and potential predators (Endler and Théry 1996). Intermediate conspicuousness may be a product of selection if the different coloration and behavioral components of conspicuousness are targeted to different predators, or if they serve as protection at different stages of a predation attempt (i.e. detection vs. recognition of unpalatability). For example, intermediate conspicuousness that results in a combination of crypsis and aposematism via distance dependence can be favored because it reduces the frequency of encounters with predators while maintaining a short range signal of unpalatability (Tullberg et al. 2005). In the intermediate phenotypes of *O. granulifera* the moderate and low brightness contrasts may reduce long range detection while the high color contrast and bold behavior may enhance recognition of unpalatability. This is because for a foraging bird, brightness contrast attracts attention, whereas the specific hue and chroma of some colors are accurately remembered (Osorio et al. 1999). Further, bold behaviors of prey, that are probably perceived at a short range, are expected to strengthen an aposematic display as more complex signals provide more reliable information (Rowe 1999). However, to our

knowledge, no studies have directly addressed the contribution of behavioral elements, other than gregariousness (e.g. Riipi et al. 2001), to the conspicuousness of prey and predator learned aversion. Such studies would be helpful to elucidate the adaptive significance of predator avoidance strategies of intermediate populations.

Importantly, both intermediate populations displayed high latency to escape like that of the green frogs. Relatively cryptic prey that cannot outrun their predators are expected to only initiate an escape in response to an imminent attack and not as soon as they detect the predator, because escaping earlier will alert a predator that may have not detected the prey yet (Broom and Ruxton 2005). Frogs from all populations tended to stop calling shortly after the predator model was in motion (B. Willink pers. obs.), but only the red frogs quickly initiated an escape response. The other three populations may avoid more predation attempts by remaining motionless, thus relying on their low detectability by means of low brightness contrast (and low exposure in the green and intermediate-green populations).

Two alternative non-adaptive explanations have been offered to account for the occurrence of intermediate phenotypes in poison-dart frogs. First, intermediate phenotypes may result from low predation pressure at these localities, enabling the exploration of the adaptive landscape via drift without a decrease in fitness (Chouteau and Angers 2012). This explanation is unlikely for *Oophaga*, because sexual selection also shapes the evolution of dorsal coloration via female preference for brighter males (Maan and Cummings 2009), males of their own color morph (Summers et al. 1999, Reynolds and Fitzpatrick 2007, Maan and Cummings 2008) and possibly through male-male aggressive interactions (Crothers et al. 2011).

Second, intermediate populations, consisting of a range of transitional color morphs, may constitute hybridization zones between red and green populations (Brusa et al. 2012) that persist due to gene flow and despite low fitness. We consider that it is possible that

assortative mating is constrained in these populations but this is not necessarily linked to reduced survivorship. As a consequence of deforestation for livestock expansion, mostly during the 1950-1960s (Kaimowitz 1996), intermediate frogs in this study occur in small forest remnants along the side of streams, which are relatively dark habitats. Intermediate frogs exhibit large variation in dorsal hue and reduced dorsal brightness. Low ambient light may impair females' color vision, facilitating inter-morph mating (e.g. Seehausen et al. 1997) and the proliferation of dorsal hues. However, the combination of reduced illumination and low dorsal brightness may also affect the rate of encounters with predators, relaxing predation risk. Additionally, human alteration has likely changed the assemblage of potential predators in intermediate populations, and may have affected particularly understory predatory birds, which are highly vulnerable to forest fragmentation (Kattan et al. 1994). Hybrids are not necessarily unfit, and they may have high fitness in perturbed habitats where they can escape competition with parental populations (Burke and Arnold 2001). Intermediate phenotypes may reflect adaptation to these changed habitat and predators; indeed, intermediate frog clay models suffer less predation than red frog models in at least one intermediate population (Willink et al. unpublished data).

Evolution of a continuum of antipredator strategies

Aposematism and crypsis are the extremes of a gradient of possible predator avoidance strategies as they represent converse solutions to the trade-off between the costs of detection by predators and the benefits of learned aversion. However, how intermediate strategies evolve from either extreme is poorly understood. Less conspicuous morphs of *O. granulifera* have evolved from bright red frogs (Wang 2011). Conspicuousness is thought to facilitate predator discrimination between toxic and palatable prey (Gamberale-Stille 2001, Darst et al. 2006) but it also incurs into higher risk of detection by predators, because only a fraction of

the predators will readily recognize the prey's noxiousness. A gradual reduction in conspicuousness would be favored if there is geographic variation in the benefits of enhanced aversion learning relative to the costs of increased encounter rates (Ruxton et al. 2009). This can occur by at least two mechanisms. First, if prey populations are exposed to different combinations of predators and predators differ in their susceptibility to prey toxins (Endler and Mappes 2004) or in the use of visual versus olfactory cues (e.g. Mochida 2011), then less conspicuous and/or more toxic phenotypes will be favored where toxin-resistant, and olfactory-oriented predators are more common. Interestingly, from South to North *O. granulifera* is decreasingly conspicuous and increasingly toxic (Wang 2011). This observation contrasts the finding that in *O. pumilio* the more conspicuous morphs for birds are also the more toxic (Maan and Cummings 2012). It is possible that reptiles, which can use olfactory cues to assess palatability of anuran prey (Li et al. 2011), play a large role for the evolution of antipredator strategies in *O. granulifera* while visual predator like birds are important for *O. pumilio*. Second, if ecological factors constrain frog density along the South-North cline the frequency of encounters with predators would be reduced correspondingly, affecting the strength of learned aversion and increasing the per capita costs of conspicuousness (Lindström et al. 2001, Comeault and Noonan 2012). Testing these hypotheses will require to 1) elucidate the identity of predators, which is currently only suspected and generalized (to birds) across populations and species of poison frogs, 2) assess how environmental variables (e.g. climate, resource availability) affect frog density and 3) assess how different signaling modalities (i.e. visual and olfactory) influence detection and aversion learning by natural predators.

Conclusions

We found evidence for a gradient of antipredator strategies along the geographic cline in coloration. Our results show that extreme color differences between red and green frogs entail clear differentiation in behavior and visual contrasts, while frogs of intermediate colorations may achieve intermediate conspicuousness through the added effects of moderate to low brightness contrast, high color contrast and different combinations of bold and cryptic behaviors. Recent studies have shown that divergent selection has played a role in geographic color diversification of poison frogs (Noonan and Comeault 2009, Brown et al. 2010, Wang and Summers 2010, Chouteau and Angers 2011). We argue that shifts in antipredator strategies towards different solutions to the predator encounter and aversion learning trade-off can involve phenotypic diversification encompassing the whole conspicuousness spectrum. An intriguing avenue for future research will be to assess the geographic variation in predator identity, physiology and behavior, to throw light upon the causes of the coupled diversification of color and behavior in poison frogs.

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provided methodological advice. Comments from two anonymous reviewers improved the final version of the manuscript. The authors declare they have no conflict of interest.

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Supporting Information

The following supporting information is available for this article:

S1. Disentangling the effects of coloration and environment on visual contrasts

(supplementary methods and results)

S2. Visual contrast analyses (supplementary results)

Figure 1. Geographic locations of the study populations of *O. granulifera* in the western lowlands of Costa Rica.

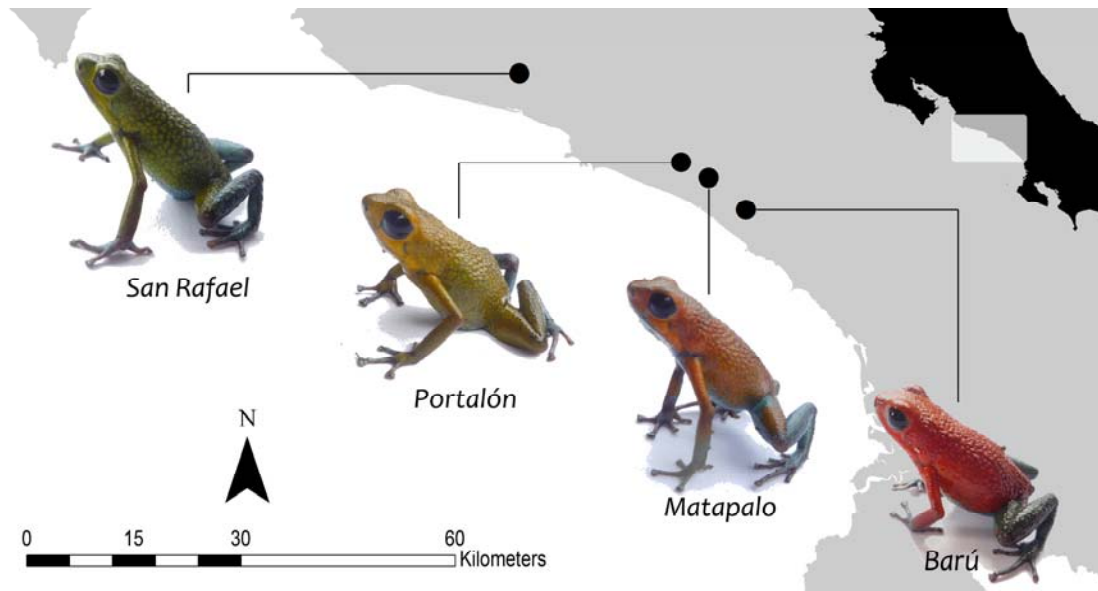


Figure 2. Reflectance spectra and color measurements (hue and chroma) of the dorsal (A, B) and ventral (C, D) skin of 99 frogs from four populations of *O. granulifera*. Reflectance spectra were averaged over all frogs found in each population. Circles represent all measured individuals and squares represent the population means.

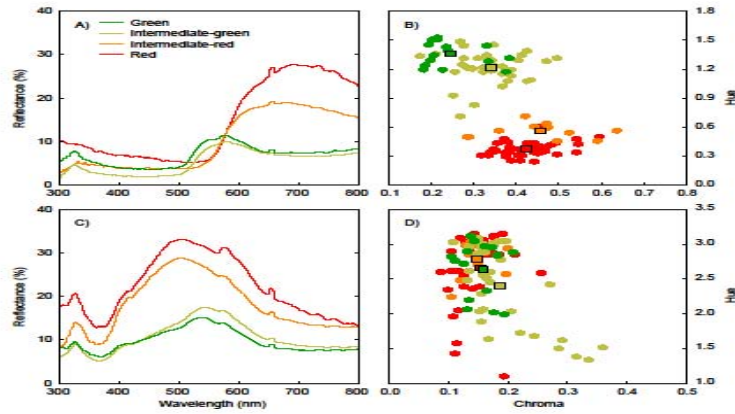


Figure 3. Mean light irradiance on the calling sites of males from four populations of *O. granulifera*.

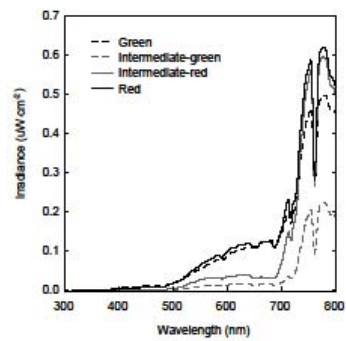


Figure 4. Color and brightness contrasts of the dorsal (A, C) and ventral (B, D) skin of *O. granulifera*, as seen by a model predatory bird. Contrasts were calculated for the most commonly used backgrounds and with the natural light conditions of each population.

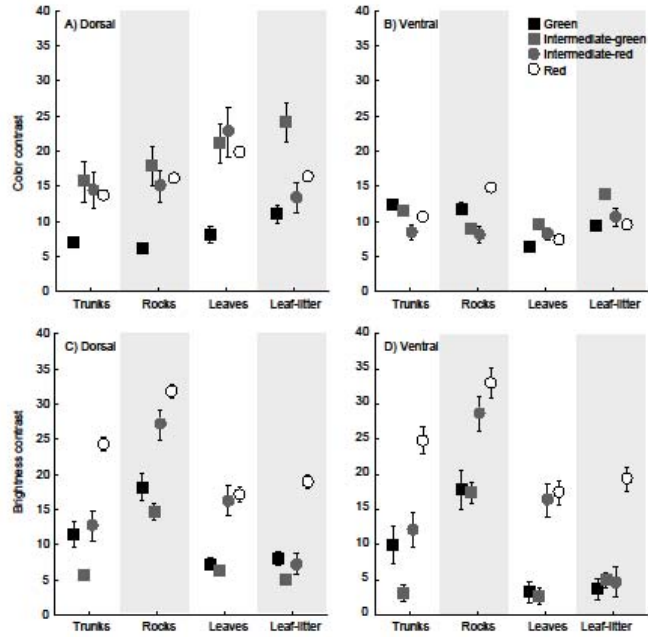


Figure 5. Mean and standard error of behavioral features of males from four populations of *O. granulifera*. **A)** Proportion of time spent calling **B)** foraging rate as the number of feeding events per hour, **C)** proportion of time spent completely inactive (i.e. not calling, foraging or moving), **D)** proportion of time moving, **E)** exposure while calling, given by the frog's position and the time spent at each position (see METHODS), **F)** flight initiation distance of calling males from an approaching bird model.

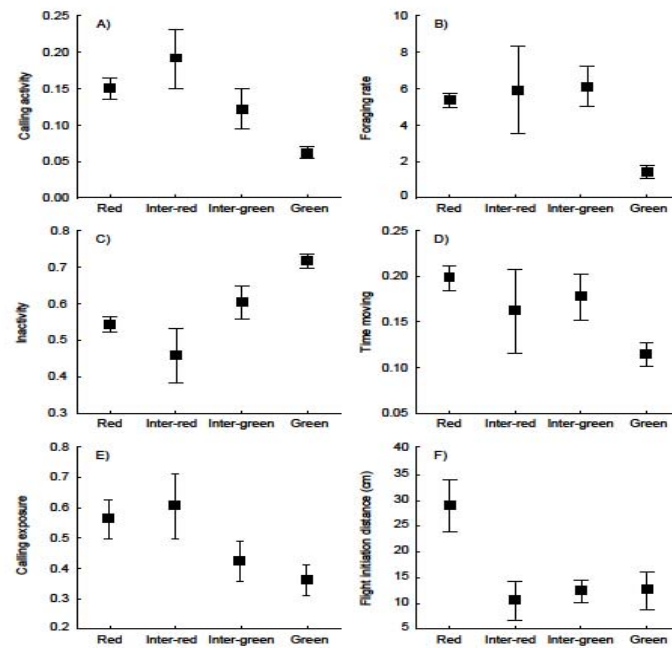


Table 1. Multivariate analysis of variance (MANOVA) examining population effects on the coloration of *O. granulifera*. To facilitate the interpretation of the overall multivariate effect an univariate analysis of variance (ANOVA) for each color variable is presented.

| | Pillai | F | DF | P value |
|----------------|------------|--------|-------|----------------|
| MANOVA | | | | |
| Dorsal | 1.09 | 18.07 | 9,285 | < 0.001 |
| Ventral | 0.53 | 6.86 | 9,285 | < 0.001 |
| ANOVA | | | | |
| <i>Dorsal</i> | Brightness | 28.97 | 3,95 | < 0.001 |
| | Hue | 417.40 | 3,95 | < 0.001 |
| | Chroma | 29.57 | 3,95 | < 0.001 |
| <i>Ventral</i> | Brightness | 22.04 | 3,95 | < 0.001 |
| | Hue | 2.68 | 3,95 | 0.051 |
| | Chroma | 5.99 | 3,95 | < 0.001 |

Table 2. P-values of post-hoc Tukey HSD comparisons of coloration elements across four populations of *O. granulifera*. Significantly different comparisons are highlighted in bold.

| | Dorsal | | | Ventral | | |
|-------------------|-------------------|-------------------|--------------|-------------------|-------------------|-----------|
| | Green | Inter-green | Inter-red | Green | Inter-green | Inter-red |
| <i>Brightness</i> | | | | | | |
| Inter-green | 0.842 | | | 0.962 | | |
| Inter-red | 0.115 | 0.008 | | 0.007 | 0.007 | |
| Red | < 0.001 | < 0.001 | 0.036 | < 0.001 | < 0.001 | 0.437 |
| <i>Hue</i> | | | | | | |
| Inter-green | 0.003 | | | 0.368 | | |
| Inter-red | < 0.001 | < 0.001 | | 0.889 | 0.121 | |
| Red | < 0.001 | < 0.001 | 0.001 | 0.996 | 0.091 | 0.920 |
| <i>Chroma</i> | | | | | | |
| Inter-green | < 0.001 | | | 0.041 | | |
| Inter-red | < 0.001 | < 0.001 | | 0.988 | 0.039 | |
| Red | < 0.001 | < 0.001 | 0.682 | 0.988 | 0.001 | 0.999 |

Table 3. Generalized linear model (GLM) mean estimates and confidence intervals for the behavioral elements of four populations of *O. granulifera*.

| | Estimate | Lower 95% CI | Upper 95% CI |
|-----------------------------------------------------------------|----------|--------------|--------------|
| <i>Advertisement call</i> (% of time spent calling) | | | |
| Green | 0.063 | 0.032 | 0.109 |
| Inter-green | 0.117 | 0.082 | 0.158 |
| Inter-red | 0.191 | 0.117 | 0.282 |
| Red | 0.148 | 0.102 | 0.204 |
| <i>Foraging rate</i> (feeding events per hour) | | | |
| Green | 1.600 | 0.664 | 3.161 |
| Inter-green | 6.133 | 4.510 | 8.105 |
| Inter-red | 5.933 | 3.379 | 9.533 |
| Red | 5.367 | 3.566 | 7.690 |
| <i>Inactivity</i> (% of time spent immobile) | | | |
| Green | 0.721 | 0.633 | 0.798 |
| Inter-green | 0.603 | 0.534 | 0.669 |
| Inter-red | 0.458 | 0.341 | 0.577 |
| Red | 0.542 | 0.457 | 0.626 |
| <i>Time moving</i> (% of minutes in which the focal frog moved) | | | |
| Green | 0.114 | 0.075 | 0.163 |
| Inter-green | 0.177 | 0.140 | 0.219 |
| Inter-red | 0.161 | 0.103 | 0.234 |
| Red | 0.198 | 0.151 | 0.251 |