

# Not all colors are equal: predation and color polytypism in the aposematic poison frog *Oophaga pumilio*

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Received: 24 March 2012 / Accepted: 29 August 2012  
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**Abstract** Aposematic organisms are not predicted to show high levels of warning signal diversity because they are expected to be under stabilizing selection to decrease costs of ‘educating’ predators about their unpalatability. However, systematic changes in warning signals (polytypism) can be expected if they represent adaptations to local predators. The aposematic strawberry poison frog (*Oophaga pumilio*) is red throughout its mainland distribution in Costa Rica and Panamá, but displays high levels of warning signal diversity in the Bocas del Toro Archipelago of Panamá. Both coloration and spot pattern vary in a polytypic sense. Sexual selection contributes to maintaining the polytypism, but little work has investigated the potential influence of predation. We used unspotted models of *O. pumilio* to determine if predation might help explain the color polytypism on Isla Colón in the Bocas del Toro Archipelago of Panamá. We tested whether attack rates differed among the red mainland morph, green/yellow Isla Colón morph, and the brown control. We found that frog color significantly predicted being attacked. The local green Isla Colón models were attacked more than foreign red or brown models. No difference in attack rate existed between red and brown control models. Our results suggest that the red mainland morph possesses a more effective warning signal, even when it is not the local morph. Honest signaling of unpalatability, neophobia, and the use of search images by local predators are potential explanations. Similarity of the brown model to other local poison frogs might explain the lower attack rate compared to previous work. The attack rate was lower on Isla Colón compared to mainland Costa Rica, which supports the hypothesis that less overall

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predation in the Bocas del Toro Archipelago may contribute to the overall warning signal diversity in *O. pumilio* there by relaxing selection for aposematic traits.

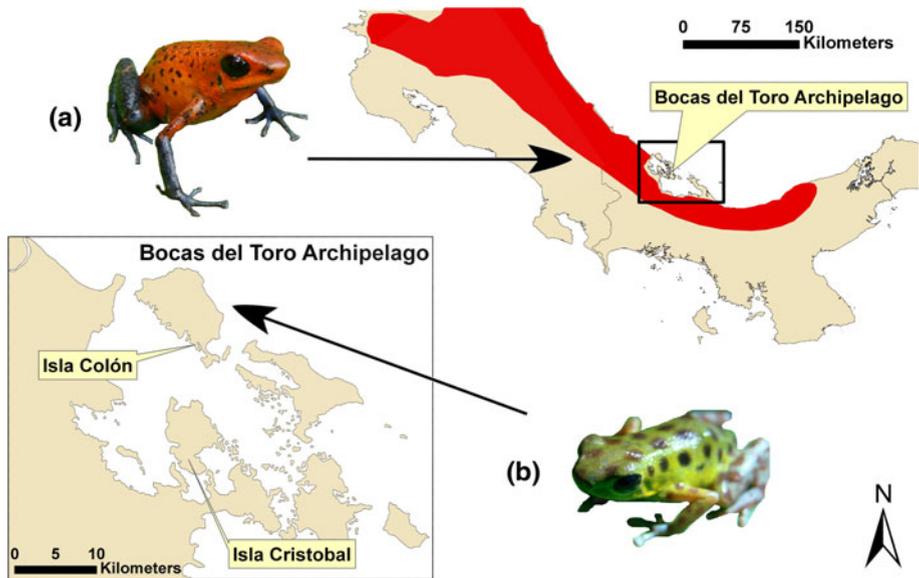
**Keywords** Color polymorphism · Conspicuous coloration · *Dendrobates pumilio* · Dendrobatidae · Model experiment · Bocas del Toro · Honest signaling

## Introduction

Natural selection typically enhances the survival and fitness of organisms, whereas sexual selection often increases conspicuousness and attractiveness at the expense of survival (Rudh et al. 2011). However, natural and sexual selection can act synergistically when organisms are aposematic (Branham and Wenzel 2003; Maan and Cummings 2008). An organism is defined as aposematic when it possesses both a defensive trait that makes it unpalatable and a warning signal to advertise its defense to potential predators (Poulton 1890; Ruxton et al. 2004; Mappes et al. 2005). The initial evolution of aposematism is often considered paradoxical because of the predicted higher predation costs associated with the original appearance of conspicuousness, which may not yet be associated with a defense (Lindström et al. 2001; Ruxton et al. 2004; Speed and Ruxton 2005). In general, predators learn to avoid aposematic prey more quickly when the diversity of warning signals is low, compared to higher signal diversity (Joron and Mallet 1998; Rowland et al. 2007). Geographic differences in warning signals are often thought to be the result of adaptations to local predators (Mallet and Barton 1989; Noonan and Comeault 2009; Chouteau and Angers 2012) or interactions between predation and other selection forces (Rudh et al. 2011). The possible explanations for geographic variation in warning signals are similar, regardless of whether the observed warning signal differences are exclusive morphotype regions (i.e., polytypisms) or many morphs co-existing in a single population (i.e., polymorphisms) alongside a dominant morph.

Typical warning colors (e.g., red and yellow) are thought to be more effective at warding off potential predators than other colors (Mappes and Alatalo 1997). Many birds possess an innate avoidance of typical warning colors (Smith 1975; Schuler and Roper 1992; Marples et al. 1998), and can also indirectly learn avoidance through observation of conspecific behaviors (Skelhorn 2011). Organisms exhibiting typical aposematic colors also tend to contrast well with the environment, which can facilitate avoidance learning in predators (Gittleman and Harvey 1980; Roper and Wistow 1986; Roper 1990). Therefore, the use of typical warning colors in many warning displays may provide protection through predator generalization, which is thought to lower overall predation costs (Mappes and Alatalo 1997; Ruxton et al. 2008). To understand fully questions surrounding the efficacy of various aposematic colors in nature (i.e., how well they deter predators), it is necessary to test different warning signals in natural conditions where the environmental context is variable, including the predator community (Endler and Mappes 2004; Lindstedt et al. 2011).

The strawberry poison frog *Oophaga pumilio* (Dendrobatidae: Dendrobatinae; Schmidt) ranges from Nicaragua through western Panamá (Savage 2002). Throughout most of its mainland range, *O. pumilio* has a reddish-orange body with blue-black legs, and small black spots on its dorsum (herein referred to as the mainland morph, Fig. 1). In the Bocas del Toro Archipelago of Panamá (Fig. 1), *O. pumilio* displays a high level of warning signal diversity. This diversity manifests itself in color polytypism across the archipelago and polymorphism in some contact zones and on some islands, most notably on Isla



**Fig. 1** A map showing the distribution of the red ‘mainland’ color morph (a) of *Oophaga pumilio* in Costa Rica and Panamá and the green ‘Colón’ color morph (b) from Isla Colón in the Bocas del Toro Archipelago of Panamá. Isla Cristobal represents one area where the mainland color morph is present in the Bocas del Toro Archipelago, in addition to the mainland. Photographs were taken by R.H.H

Bastimentos (see plates in Myers and Daly 1983; Rudh et al. 2007; Saporito et al. 2007a). Over 15 color morphs are present in the archipelago and include disparate colors such as yellow, red, blue, lime green, and bronze among others, with different morphs occurring at times on the same island (Siddiqi et al. 2004). Dorsal spots are also present in some morphs in the Bocas del Toro Archipelago, although the size and number of spots varies depending on the specific morph. Herein, we use the phrase “warning signal diversity” to allow general discussion of geographic patterns where there may be variable, polytypic, and polymorphic characters in the warning signal within the same population, or region as a whole. Brown et al. (2010) found evidence that divergence in dorsal color within the Bocas del Toro Archipelago was occurring more quickly than would be predicted under a neutral process, such as genetic drift. Underlying factors that might explain the warning signal diversity in the archipelago include sexual selection (Summers et al. 1997; Maan and Cummings 2008; Tazzyman and Iwasa 2010; Rudh et al. 2011) and predation (Siddiqi et al. 2004; Reynolds and Fitzpatrick 2007; Saporito et al. 2007b; Maan and Cummings 2008; Brown et al. 2010; Wang and Summers 2010; Rudh et al. 2011; Maan and Cummings 2012). Previous work has shown that the red morph of *O. pumilio* in Costa Rica is avoided by predators (Saporito et al. 2007b), and that the black spotting pattern on the same morph does not influence the attack rate (Hegna et al. 2011). However, no work has experimentally investigated the role of predation with respect to the warning signal diversity within *O. pumilio* across the archipelago.

The degree of conspicuousness varies among color morphs in the Bocas del Toro Archipelago (Siddiqi et al. 2004). Using vision modeling, Siddiqi et al. (2004) demonstrated that the most visible color to conspecifics was the orange-red morph from Isla Solarte, and the most visible color to birds was the red-bodied morph from Almirante. The

Almirante morph is most similar in color to that of *O. pumilio* throughout most of its mainland range. In general, red colors ( $\sim 640\text{--}700$  nm) are predicted to be the most conspicuous colors in forests (Endler 1993; Wheelwright and Janson 1985; Alves-Costa and Lopes 2001; Osorio and Vorobyev 2005). The conspicuousness of the red mainland morph may be further enhanced by the dark legs that outline and contrast with the body when the frog is in a resting position. In general, color elements that outline the body of an organism increase conspicuousness (Cuthill et al. 2005). Maan and Cummings (2012) recently found that conspicuousness correlated with toxicity in *O. pumilio*, strengthening the hypothesis that coloration serves as an honest signal of unpalatability (but also see Daly and Myers 1967). A combination of the conspicuousness to avian predators and overall predicted higher visibility in complex environments suggests the red mainland morph of *O. pumilio* (and other similar morphs) may possess a more effective warning signal than other frogs in the complex. However, whether more toxic and conspicuous morphs are avoided by predators when compared to the less toxic and less conspicuous morphs is unknown (Maan and Cummings 2012).

In the current study, we examine the hypothesized role of predation in shaping or maintaining the polytypism of *O. pumilio* in the Bocas del Toro Archipelago. We conducted a predation experiment on Isla Colón using unspotted models of *O. pumilio* to test the hypothesis that the red mainland morph bears a more effective warning signal than the green Isla Colón morph (Fig. 1). We predicted that the mainland color morph would experience a lower attack rate than that of the Isla Colón color morph. We also compared attack rates on Isla Colón in the Bocas del Toro Archipelago, to data from mainland Costa Rica where previous studies using clay models were completed. Reduced predation pressure may provide an additional explanation for the increased warning signal diversity observed in *O. pumilio* in the Bocas del Toro region, especially since increased predation pressure is predicted to decrease warning signal diversity (Puurtilinen and Kaitala 2006; Rudh et al. 2007; Chouteau and Angers 2012).

## Methods

Fieldwork was conducted from 23 July to 30 August 2008 on Isla Colón in Bocas del Toro, Panamá. The methods used in the present study are similar to those used in Hegna et al. (2011). Spots were not used on frog models in the present study in an attempt to isolate the effect of color. Models made of Sculpey III<sup>®</sup> polymer clay were constructed with pre-manufactured molds made from preserved frog specimens to ensure consistent body shape across all models used for the study (see Hegna et al. 2011 for additional details; Fig. 2). Polymer clay models record identifiable marks left by an attack from predators and therefore represent one manner in which to estimate natural predation rates (e.g., Brodie 1993; Saporito et al. 2007b; Hegna et al. 2011).

We constructed models to mimic the dorsal color of *O. pumilio* (Schmidt) from two populations: (1) Isla Colón and (2) the mainland of Costa Rica. Models of small non-poisonous frogs of the genus *Craugastor* (Cope) commonly found in the leaf litter throughout forests in lower Central America were also constructed as controls. *Oophaga pumilio* (Schmidt) on Isla Colón have a green dorsal color with yellow flanks, large spots on the dorsum and brown-orange legs, whereas *O. pumilio* (Schmidt) on the mainland have reddish-orange bodies with blue legs. Members of the genus *Craugastor* (Cope) are often brown in body color (Savage 2002). Studies of predation on poison frogs typically use visual comparisons to match colors (e.g., Saporito et al. 2007b; Noonan and Comeault

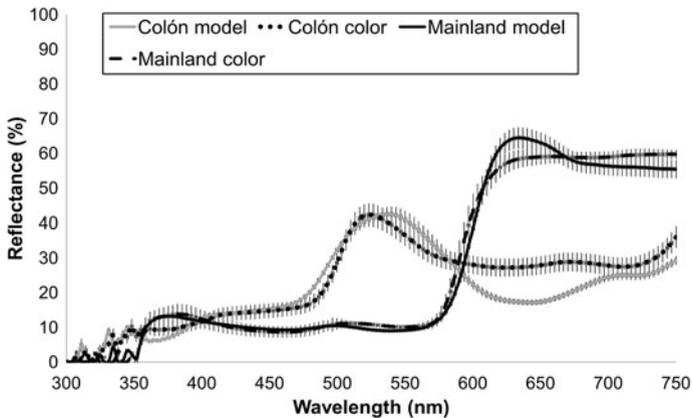


**Fig. 2** The three frog model types used in Panamá: The Isla Colón color morph (*left*), cryptic model (*middle*), and mainland color morph (*right*). Photographs were taken by R.H.H

2009; Chouteau and Angers 2012) because dendrobatid coloration mostly lacks UV reflectance, which makes by-sight comparisons easier (Noonan and Comeault 2009; see also Summers et al. 2003). However, we attempted to improve upon these methods to better ensure frog model color accuracy. To match our model colors to the actual frog color, we used color frequency data collected in 2006 to determine the dominant color for the Isla Colón morph and mainland morph (Donnelly and Saporito, unpublished data: Online Auction Color Chart, <http://www.onlineauctioncolorchart.com/>). At each location, *O. pumilio* were collected and brought back to the field station laboratory where both their dorsal and ventral colors were matched against the color chart by eye under similar light conditions. These data allowed us to determine the most frequently encountered dorsal color in each location. The most frequently encountered green dorsal color on the Isla Colón morph was color 91 ( $n = 3$  out of 15, though others were adjacent shades) and the dominant yellow ventral color was 896 ( $n = 10$  out of 15). The red dorsal color for the mainland morph was color 649 ( $n = 36$  out of 101). Frogs of the genus *Craugastor* (Cope) were represented by a brown color previously used in other studies of aposematism in *O. pumilio* (Schmidt) and *Dendrobates tinctorius* (Cuvier) (Saporito et al. 2007b; Noonan and Comeault 2009). Sculpey III<sup>®</sup> polymer clay of different colors was then mixed to create matches to the dominant color for each morph. A Sharpie<sup>®</sup> permanent marker was used to create eyes for the models.

On the basis of previous clay frog model experiments, the majority of predators of adult *O. pumilio* (Schmidt) appear to be birds (Saporito et al. 2007b) that have tetra-chromatic color vision (Osorio and Vorobyev 2005), whereas humans have tri-chromatic color vision. To objectively ensure our clay mixes accurately matched the most frequently encountered color in each population, according to the color chart, we determined the final color mixture with a spectroradiometer (PP SYSTEMS Unispec-SC; Fig. 3). We measured the color directly from the color chart, compared it with each clay mixture, and adjusted the mixtures accordingly. We were also able to visually compare the general shape of our reflectance curves to those reported in Summers et al. (2003). Previous studies found that *O. pumilio* (Schmidt) do not reflect UV light (Summers et al. 2003). Therefore, we verified that our clay mixtures and permanent marker used to create eyes did not reflect UV light.

We established 58 transects in a mixture of primary and secondary forest on privately owned land away from heavily populated areas on the island. Each transect was 105 m in length and had seven red models, seven green models, and seven brown models (21 models total). Models were spaced 5 m apart and the order of model placement was determined by a die roll (with two numbers referring to one color type), except that no more than two models of the same color were placed consecutively along each transect (Saporito et al. 2007b;



**Fig. 3** The reflectance spectra showing how the clay mixture for each model type's dorsal color matched against the most frequently encountered dorsal color, measured from the color chart. *Error bars* represent standard error

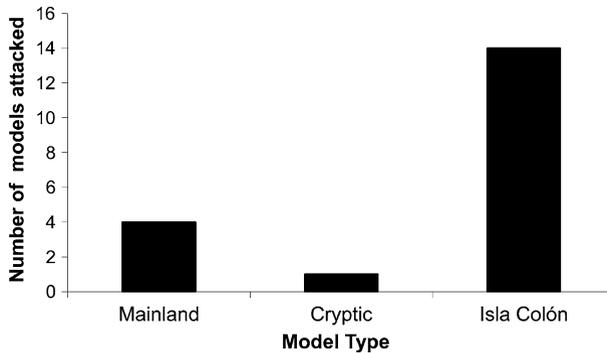
Hegna et al. 2011). To reduce any effects of crypsis, half of the 58 transects contained models placed on 9.5 cm × 10 cm white “Rite-in-the-Rain”<sup>®</sup> pieces of paper. A total of 1,218 model frogs were used in this experiment ( $n = 406$  for each model type).

#### Predation determination and statistical analysis

After 48 h, models were collected and scored as either attacked or not attacked (Brodie 1993; Saporito et al. 2007b; Hegna et al. 2011). Missing models were excluded from the final analysis (though their inclusion did not change any results). Attacked models were tagged, photographed, and stored. Bird attacks were determined by a clear “U” or “V” shaped beak mark left on the model (see Hegna et al. 2011). Although bite marks from arthropods and mammals can also appear on models, they were excluded from our final statistical analysis, as these organisms likely do not represent actual predators and do not always possess color vision.

We used binary logistic regression to determine if variation in attack rate was explained by frog model color, background type (paper or leaf litter), and the interaction between the two variables. The final model included all variables except the interaction term, as it was not significant in the initial test ( $p = 0.96$ ).

We also compared avian attack rates from Isla Colón with the average attack rate from two other predation studies completed in 2008 on *O. pumilio* (Schmidt) in Costa Rica at La Selva Biological Research Station. They included: (1) the effectiveness of spots in the warning signal of *O. pumilio* (Schmidt) (see Hegna et al. 2011), and (2) a study of the effect of density on predation (Hegna 2009). The attack rates in both studies completed in 2008 were not significantly different ( $p = 0.876$ ), thus the data were combined to create an average attack rate to better represent predation from that site. Although some red mainland morph models used in Hegna et al. (2011) contained small spots, these did not influence attack rates in that study and did not need further consideration when deriving the average attack rate from both studies. Models from the density experiment in Hegna (2009) were the same color as the red mainland morph models used in the present study. A G-test was used to determine if our estimated predation pressure was greater on the mainland



**Fig. 4** The number of attacks on different model types on Isla Colón Panamá

(using the average of the two studies) compared to that on Isla Colón. Attack rates on the two individual color treatments used on Isla Colón (red mainland morph, and green/yellow Colón morph) were also compared to the attack rate observed on the red mainland morph in Costa Rica.

## Results

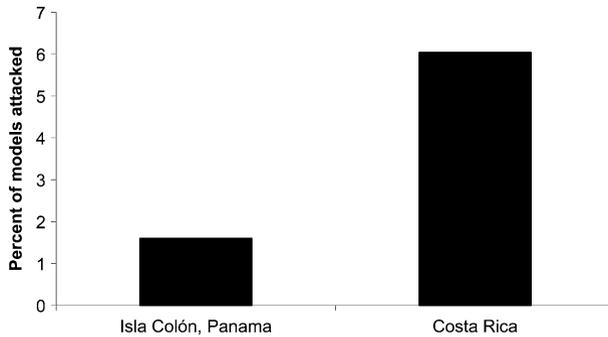
Of the 1,218 models, a total of 91 models were attacked in forests on Isla Colón. Of these, 19 (21 % of attacks) were attacked by birds. Only 18 (1.5 % of all models) were not recovered and were scored as missing. When attacks by other potential attackers were analyzed we found no differences in predation on the different model types (analyzing for mammal, insects, and unknown attackers).

### Analysis of avian predation- Panamá

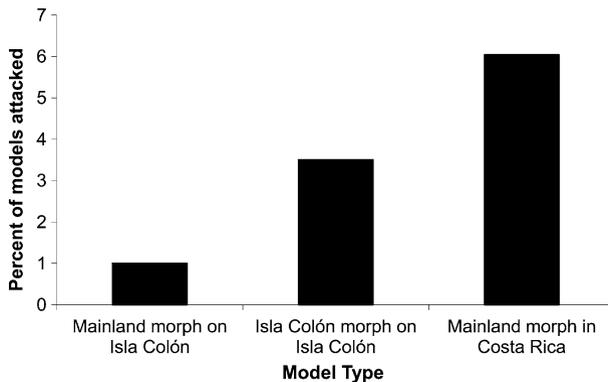
Frog color was a significant predictor of being attacked ( $p = 0.005$ , Wald  $X^2 = 10.4$ ,  $df = 2$ ; Fig. 4). The green Isla Colón morph was 3.6 times more likely to be attacked than the red mainland morph ( $p = 0.025$ , odds ratio = 3.6,  $CI_{95\%} = 1.2-11.0$ , Wald  $X^2 = 5.0$ ,  $df = 1$ ). The green Isla Colón morph was also 14.5 times more likely to be attacked than the brown cryptic morph ( $p = 0.01$ , odds ratio = 14.5,  $CI_{95\%} = 1.9-110.6$ , Wald  $X^2 = 6.6$ ,  $df = 1$ ). However, probability of being attacked did not differ between the cryptic brown morph and red mainland morph ( $p = 0.21$ , odds ratio = 0.3,  $CI_{95\%} = 0.03-2.2$ , Wald  $X^2 = 1.5$ ,  $df = 1$ ). The background type (leaf litter or white paper) the model was placed on also did not significantly predict being attacked ( $p = 0.49$ , odds ratio = 1.4,  $CI_{95\%} = 0.6-3.5$ , Wald  $X^2 = 0.5$ ,  $df = 1$ ).

### Predation rate comparison

The average avian attack rate measured from mainland color morphs of *Oophaga pumilio* in Costa Rica at the La Selva Biological Station during the same summer was 6 % (93/1540 models; Hegna 2009; Hegna et al. 2011). The overall estimate of the avian attack rate on Isla Colón (all three model types included) was 1.6 % (Fig. 5). A comparison of the



**Fig. 5** The overall percentage of models attacked in Costa Rica and the current study on Isla Colón, Panamá



**Fig. 6** Avian attacks on red mainland and green Colón models on Isla Colón compared to the average attack rate on red mainland models observed in the two studies from Costa Rica

attack rate on Isla Colón with Costa Rica found the attack rate on Isla Colón to be significantly less than in Costa Rica ( $p < 0.001$ , G-statistic = 38.9,  $df = 1$ ).

When we compared the specific attack rate of the two model types of *O. pumilio* morphs used on Isla Colón with the attack rate on red models used in the Costa Rica studies the results did not change (Fig. 6). On Isla Colón 1 % of all red models and 3.5 % of green models were attacked. Green models from Isla Colón were attacked significantly less than red models from Costa Rica (G-statistic = 4.6,  $p = 0.032$ ,  $df = 1$ ). Red mainland models on Isla Colón were also attacked significantly less than red mainland models in Costa Rica (G-statistic = 23.6,  $p < 0.001$ ,  $df = 1$ ).

## Discussion

The results of our study on Isla Colón suggest that the level of bird predation varies according to color in the Bocas del Toro Archipelago of Panamá. The local Isla Colón morph was attacked more by birds than the non-local mainland morph, which appeared more effective at deterring predators. Our findings differ from those of Noonan and

Comeault (2009), who found that the local morph of *D. tinctorius* was attacked less than a non-local morph. Instead, our results support a second possible outcome not previously demonstrated for experiments exploring locally adapted warning signals. We show that some signals are potentially more effective regardless of the local signal. One possible explanation for the difference in our results from Noonan and Comeault (2009) is the recent evidence suggesting that the different warning signal colors of *O. pumilio* function as honest signals of unpalatability (see Maan and Cummings 2012) in Bocas del Toro, Panamá.

Honest signaling within aposematic species remains a highly debated topic (Blount et al. 2009). For instance, Darst et al. (2006) showed signal strength in some members of the poison frog genus *Epipedobates* (Myers) to be inversely related to their toxicity. Wang (2011) also showed that conspicuous coloration and toxicity were inversely related in *Oophaga granulifera* (Taylor), a dendrobatid closely related to *O. pumilio*. However, another study demonstrated a link between toxicity and coloration in marine opisthobranchs (Cortesi and Cheney 2010). Our study offers supportive evidence for honest signaling in *O. pumilio* because the pattern of attacks was correlated with observed toxicity and conspicuous measures (Siddiqi et al. 2004; Maan and Cummings 2012). In our study, models of the less toxic and less conspicuous green Isla Colón morph were attacked more than models of the red mainland morph. Daly and Myers (1967) found that toxicity did not correlate with coloration in *O. pumilio*, though it is important to note that color was a qualitative consideration in their study. Because toxicity is acquired from dietary sources in dendrobatids that vary temporally and spatially (Saporito et al. 2007a), it is important to consider temporal resource variation in discussions of honest signaling in poison frogs. Likewise, predation in the archipelago may also vary temporally. Therefore, we agree with Maan and Cummings (2012) that further information regarding the ecological and physiological aspects of *O. pumilio* is needed to verify honest signaling and relate the system to theory in a realistic fashion. Additional work will hopefully also determine the extent to which our observations supporting honest signaling hold for other color morphs.

Neophobia, fear of a novel item, may have contributed to a lower attack rate on the red mainland color morph as well, particularly if the birds were less familiar with red frogs (Marples et al. 1998; Mappes et al. 2005). However, the close proximity of Isla Colón to other islands with red frogs (Almirante and Isla Cristobal see Fig. 1; also see Summers et al. 2003; Siddiqi et al. 2004) raises the possibility that some predators attacking *O. pumilio* have previously encountered red frogs. Although some species of birds may visit adjacent islands, others are less likely to move across water (pers. com. James Roper). Knowledge about specific predators is needed and will allow more detailed investigation into the behavioral underpinnings of the aversion we observed in our study.

A lower attack rate on the red mainland morph could be the product of local predators possessing a pre-existing search image for the green Isla Colón morph. Predators foraging with a 'search image' typically have an increased ability to detect familiar prey (Punzalan et al. 2005). The formation of search images by predators is mostly considered an adaptation to locating cryptic prey that are hard to detect from their surroundings by focusing attention on a limited set of search criteria (Punzalan et al. 2005). However, studies show that conspicuous or distinctive prey items are not undetectable to birds foraging with a pre-formed search image (see Bond and Riley 1991; Blough 1992; Reid and Shettleworth 1992), which makes it less likely that aposematic organisms bearing conspicuous or distinctive warning signals would be ignored. The red mainland morph is more conspicuous to birds than the green Isla Colón morph (Siddiqi et al. 2004, Maan and Cummings 2012), and the color red is generally predicted to be one of the most conspicuous colors in forest

environments (Endler 1993; Wheelwright and Janson 1985; Alves-Costa and Lopes 2001; Osorio and Vorobyev 2005). Thus, the increased conspicuousness of the red mainland morph likely limits the possibility that fewer attacks on the red mainland morph were the result of reduced detection by predators operating with a biased search image. Additionally, previous studies looking at locally adapted warning signals in dendrobatids show the unfamiliar foreign morph to be attacked more than the local morph (Noonan and Comeault 2009, Chouteau and Angers 2012), which provide some evidence that foreign aposematic morphs are generally detected.

A greater rate of attack on the local green Isla Colón morph might also be explained by the fact that we did not include spots on the dorsal surfaces of the clay models of *O. pumilio*. Frogs from Isla Colón are spotted, though we did not include spots in an attempt to only test the importance of base color alone. Sparsely spotted individuals and individuals with very small spots, though, do exist naturally and should limit any effect of not including spots on our models (13/66 individuals observed in 2008). While black contrasting patterns may be important in the displays of some aposematic organisms, mainly through increased recognition by predators (Aoki et al. 2000), recent work suggests that contrasting patterns are less important than color (Aronsson and Gamberale-Stille 2008; Hegna et al. 2011). Furthermore, Hegna et al. (2011) found the spot pattern of *O. pumilio* from northeastern Costa Rica, characteristic of most mainland frogs, did not affect attack rates. However, the effect of the large spot pattern present on the dorsum of some *O. pumilio* color morphs is untested in the Bocas del Toro Archipelago and is worthy of additional study.

Differences in habitat use by Isla Colón populations, compared to mainland populations may be an additional explanation for increased attack frequency on the Isla Colón morph. Specifically, *O. pumilio* on Isla Colón appear more arboreal than other populations in Bocas del Toro and on the mainland (Daly and Myers 1967). Isla Colón frogs also possess a bright yellow ventral base color. If *O. pumilio* on Isla Colón are more arboreal than those from other locations, it is possible that the yellow ventral region of these frogs may act as part of the aposematic signal and certainly would be more readily seen by potential avian predators than our experimental design allowed. However, placing the models on leaf litter, as in our experiment, is not out of context, as many *O. pumilio* are commonly observed moving over the leaf litter on Isla Colón.

The low attack rate on our brown control models is of particular interest because it is counter to the results of several other studies on aposematic organisms (e.g., Brodie 1993; Saporito et al. 2007b). In a recent study of predation on the dendrobatid frog *D. tinctorius*, Noonan and Comeault (2009) also found that the avian attack rate did not differ between their brown control morphs and aposematic morph. Increased or equal survival of control morphs has also been documented in studies of aposematism and mimicry of tropical butterflies, in which control morphs experienced equivalent attack rates to warningly colored individuals (Brower et al. 1964; Cook et al. 1969; Ruxton et al. 2004). Waldbauer and Sternburg (1975) proposed that one reason for the results obtained by Brower et al. (1964) and Cook et al. (1969) was because of the potential resemblance of the control butterflies to another unpalatable butterfly species. Likewise, one possible explanation for the equal attack rates between the brown cryptic frog models and red mainland frog models in our present study might be the similarity in shape and color of our brown models to other poison frogs present on Isla Colón. Unlike the study site in Costa Rica (La Selva Biological Reserve), Isla Colón appears to have a higher abundance of darkly colored poison frogs in the genus *Phyllobates* (Duméril and Bibron), *Colostethus* (Cope), *Allobates* (Zimmermann and Zimmermann), and *Silverstoneia* (Grant et al. 2006) (pers. obs.). Though *Colostethus*

is a dully colored aromobatid (a sister group to the dendrobatids), Poulin et al. (2001) observed that the entire genus was avoided in their survey of 1,086 bird stomachs along the Panamá Canal (the genus *Colostethus* was partitioned into several genera including *Allobates* and *Silverstoneia*). Endler and Mappes (2004) raised the point that a warning signal does not need to be conspicuous, but only recognizable by predators per the original definition of aposematism used by Poulton (1890). Recent work showing the importance of viper head shape in deterring predators supports the importance of non-conspicuous aspects of aposematic organisms being important via recognition for warning predators (Valkonen et al. 2011). Therefore, it is possible that the characteristic dendrobatid shape of the brown model, used in the context of other semi-abundant dark poison frogs on Isla Colón, was enough to convince predators that the model was an unpalatable poison frog. Similarly, brown and red mainland models may appear more alike if potential predators forage in low light conditions, which may result in predators avoiding the brown model because it begins to look too similar to red.

The avian attack rate on Isla Colón, Panamá was significantly less than that found on the mainland in Costa Rica. With less predation pressure on aposematic organisms, the strength of selection on warning signal appearance would probably decrease as one numerical model implies (Puurtilinen and Kaitala 2006). Decreased predation pressure is also thought to be an over-arching explanation for increased diversification in aposematic taxa when compared to closely related non-aposematic sister groups in phylogenetic comparisons (Przeczek et al. 2008). Our observation of lower attack rates on one island in the Bocas del Toro Archipelago (where warning signal diversity is high) compared to the attack rates in Costa Rica (where less warning signal diversity exists) supports the implications of the model proposed by Puurtilinen and Kaitala (2006). Additional studies across the Bocas del Toro landscape are required to determine if low attack rates are characteristic of other islands in the archipelago and adjacent mainland. In the case of *Oophaga pumilio*, release from predation pressure may have permitted the evolution of color polytypism and higher warning signal diversity in conjunction with other factors such as, sexual selection or genetic drift via vicariant geologic events (see Anderson et al. 2002 for a summary of the geological history of the Bocas del Toro Archipelago). Noonan and Comeault (2009) also obtained an avian attack rate of 3.1 % on aposematic forms of *D. tinctorius* (2.3 % total attack rate), another dendrobatid frog that displays a high degree of color polymorphism in French Guiana. Furthermore, Chouteau and Angers (2012) observed decreased attack rates in areas of higher phenotypic diversity in another poison frog, *Ranitomaya imitator* (Schulte). The lower attack rate observed in French Guiana, and the results from Chouteau and Angers (2012) provide additional support for the hypothesis that reduced predation pressure might be a general factor that facilitates greater warning signal diversity in dendrobatids. Other factors undoubtedly do contribute to the warning signal diversity in *O. pumilio* and other species, but certainly differences in overall predation pressure are intriguing and warrant further study as they appear to align with model predictions.

One counterargument to the hypothesis that decreased predation pressure would permit the observed higher warning signal diversity in *O. pumilio* is that other dendrobatids present on the islands do not show the same level of diversity in their warning signal (Summers et al. 2003; Rudh et al. 2007). A genetic pre-disposition toward phenotypic variability (including warning signal diversity) in *O. pumilio*, though, may be at least one explanation. Throughout its mainland range *O. pumilio* displays phenotypic variability (unpublished data RA Saporito and MA Donnelly; see also Wang and Shaffer 2008), despite higher levels of gene flow than in the Bocas del Toro Archipelago (Wang and Summers 2010). Other members of the genus *Oophaga* also possess highly diverse

warning signals, namely *Oophaga histrionica* (Berthold) and *Oophaga sylvatica* (Funkhouser). The combination of female parental care and sexual selection in *O. pumilio*, unlike other dendrobatids that display male parental care, might contribute to the differences in warning signal diversity as well (Summers 1997).

Geographic differences in predator communities may also be associated with the diversity in coloration of *O. pumilio* in the Bocas del Toro Archipelago (Reynolds and Fitzpatrick 2007). Differently shaped beak imprints left in the models from Costa Rica (Hegna et al. unpublished data) compared to Isla Colón in the present study suggests the possibility that predator communities may differ between the mainland in Costa Rica and Isla Colón. Endler and Mappes (2004) developed a model that suggested variability in predator communities might allow for decreased levels of conspicuousness in the warning signal, which could permit more variation in color. Additionally, net selection by a group of predators can be less selective than by single predators (Exnerová et al. 2003; Lindstedt et al. 2011). Whether differences in predator communities contribute to polytypism in *O. pumilio*, though, remains unknown.

Understanding warning signal diversity in aposematic organisms continues to be a main focus in evolutionary biology as a way of studying natural selection and interactions among selective forces (Nokelainen et al. 2012). Our findings support the hypothesis that predation is a selective force acting on the observed polytypism in *Oophaga pumilio*, and substantiate recent work suggesting that coloration functions as an honest signal toward predators (see Maan and Cummings 2012). Additional work is needed to verify and understand honest signaling in *O. pumilio*. Our results do not support the hypotheses that all color morphs of *O. pumilio* are equally protected from predation (see Siddiqi et al. 2004). Lower overall predation pressure in the Bocas del Toro Archipelago may relax selection for optimality in the warning signal to the point where significant differences in predation are less important from an evolutionary standpoint. The role of predation and the interplay between sexual selection and predation in *O. pumilio* will continue to provide a fascinating arena in which to study natural selection. Our study shows the potential importance of predation pressure in dendrobatid frogs and provides evidence that predation can be a factor shaping warning signal diversity through a variety of mechanisms.

**Acknowledgments** We thank the Smithsonian Tropical Research Institute (STRI) Bocas del Toro field station and the Institute for Tropical Ecology and Conservation (ITEC) for valuable logistical support. This work was completed with official permission from the Autoridad Nacional del Ambiente (ANAM permit number SE/A-68-08). Jonathan R. Hegna and Humberto Vlades provided assistance in field work preparations. Janna Goldrup's work at ITEC contributed, in part, to the idea for this study. The manuscript was improved by helpful comments from Johanna Mappes, Craig Guyer, Mike Heithaus, Frank Hensley, Natalie Hyslop, Thomas R. Jones, Monica Isola, Seiichi Murasaki, A. Justin Nowakowski, Kelsey Reider, Janne Valkonen, Steven Whitfield, and the herpetology group at John Carroll University. The manuscript also benefitted from suggestions by editors John Endler and Manuel Leal, along with the anonymous reviewers. We thank Mikael Puurtinen for discussions about the implications of their numerical model. Kenneth G. Gerow graciously provided statistical consulting. Funding was provided by the American Society of Ichthyologists and Herpetologists, the Judith Parker Travel Fund, Organization for Tropical Studies, Greater Cincinnati Herpetological Society, and the Chicago Herpetological Society. A National Science Foundation Postdoctoral Research Fellowship partially supported RAS. This paper is contribution 237 to the program in Tropical Biology at Florida International University.

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