

No evidence for differential survival or predation between sympatric color morphs of an aposematic poison frog

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Received: 30 September 2012 / Accepted: 23 February 2013
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Abstract Because variation in warning signals slows down the predator education process, aposematic theory predicts that animal warning signals should be monomorphic. Yet, warning color polytypisms are not uncommon in aposematic species. In cases where warning signal variants are separated geographically, adaptation to local predators could explain this variation. However, this cannot explain the persistence of sympatric polymorphisms in aposematic taxa. The strawberry poison frog (*Oophaga pumilio*) exhibits both allopatric and sympatric warning color variation in and around the Bocas del Toro archipelago of Panama. One explanation that has been proposed for the rapid diversification of *O. pumilio* coloration in this archipelago is low predation; if island populations have few predators, stabilizing selection would be relaxed opening the door for diversification via selection or genetic drift. Using a combination of mark-recapture and clay model studies, we tested for differences in survival and predation among sympatric red and yellow color morphs of *O. pumilio* from Bastimentos Island. We found no evidence for differential survival or predation in this population, despite the fact that one morph (red) is more common and widely distributed than the other (yellow). Even in an area of the island where the yellow morph is not found, predator attack rates were similar among morphs. Visual modeling suggests that yellow and red morphs are distinguishable and conspicuous against a variety of backgrounds and by viewers with different visual systems. Our results suggest that general avoidance by predators of red and yellow, both of which are typical warning colors used throughout the animal kingdom, may be contributing to the apparent stability of this polymorphism.

Electronic supplementary material The online version of this article (doi:[10.1007/s10682-013-9636-0](https://doi.org/10.1007/s10682-013-9636-0)) contains supplementary material, which is available to authorized users.

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Keywords Color polymorphism · Polytypism · Aposematism · Bocas del Toro · *Dendrobates pumilio* · Predation · Survival

Introduction

Aposematism, the possession of both a defensive trait that renders an organism unpalatable and a warning signal that advertizes this defense to would-be predators (Poulton 1890), occurs in a number of animal taxa (Ruxton et al. 2004). The effectiveness of this defensive strategy depends upon the ability of predators to associate the warning signal with the unprofitability of prey (Mappes et al. 2005). Because predators often learn more quickly to avoid aposematic prey when the diversity of warning signals they present is low, theory predicts that aposematic species should be under strong stabilizing selection, resulting in monomorphism (Joron and Mallet 1998). The presence of polytypic warning signals in defended taxa, therefore, presents somewhat of an evolutionary paradox due to the presumably higher cost of predator education (Summers et al. 1997; Joron and Mallet 1998). Nevertheless, in some taxa such variation in warning signals is not uncommon.

A number of poison frogs in the family Dendrobatidae exhibit this puzzling combination of chemical defense and polytypic warning signal (e.g., Summers et al. 1997; Noonan and Comeault 2009; Chouteau et al. 2011). In most cases, warning signal variants tend to be geographically isolated such that differential adaptations to local predator communities could explain the diversity of warning coloration (Noonan and Comeault 2009; Chouteau and Angers 2011; Hegna et al. 2012). However, in the rare cases where two or more morphs exist in sympatry, other explanations for the evolution and persistence of multiple warning phenotypes must be considered (Chouteau and Angers 2012).

The strawberry poison frog (*Oophaga pumilio*, formerly *Dendrobates pumilio*, see Brown et al. 2011) exhibits an amazing variety of color and pattern variants (hereafter, color morphs) on the islands of Bocas del Toro and the adjacent mainland of Panama. Like many dendrobatid frogs, *O. pumilio* defends itself from would-be predators by sequestering alkaloids in its skin (Daly and Myers 1967; Saporito et al. 2006). Field studies using clay models have supported the idea that bright colors serve an aposematic function in this species (Saporito et al. 2007; Hegna et al. 2012), suggesting a role for natural selection in the evolution of phenotypic variation. However, studies of female mate preferences (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008, 2009; Richards-Zawacki and Cummings 2011) and evidence of assortative mating in the wild (Richards-Zawacki et al. 2012) suggest that coloration is also under sexual selection.

Although most *O. pumilio* variants are allopatric, there are a few populations where two or more distinctive color morphs occur in sympatry, providing an opportunity to evaluate the roles of various selective forces in the evolution of warning signal diversity. One such population exists on the northwest tip of the island of Bastimentos, where yellow and red morphs co-occur. This polymorphism appears to have a simple Mendelian pattern of inheritance (Richards-Zawacki et al. 2012) and exists in contrast with the rest of the island, where populations are red to red–orange in color and variation (mostly in the size and number of black spots) tends to occur allopatrically. Females from the polymorphic population prefer to court with males of their own morph (Richards-Zawacki and Cummings 2011) and there is evidence for color assortative mating by red (but not yellow) frogs in the wild (Richards-Zawacki et al. 2012), suggesting a role for sexual selection in

generating or maintaining this polymorphism. However, the role of natural selection remains unclear.

In both polymorphic and monomorphic regions of Bastimentos, skin alkaloid profiles are less variable within than among populations, suggesting similar levels of unpalatability among sympatric morphs (Saporito et al. 2006). However, the red morph is (a) more widely distributed, (b) more abundant than the yellow morph throughout most of the polymorphic region (Richards-Zawacki and Cummings 2011), and (c) shows a stronger tendency to mate assortatively in the wild than the yellow morph (Richards-Zawacki et al. 2012). Given this, we hypothesized that yellow frogs are at a selective disadvantage and should decrease in frequency over time due to higher predation, the tendency of the red morph to mate assortatively, or both. Using a combination of mark-recapture and clay model experiments, we tested for differences in survival, changes in frequency over time, and predation among red and yellow *O. pumilio* from the polymorphic region of Bastimentos. We predicted that the less common yellow morph would exhibit lower survival and a downward trend in frequency in the mark-recapture study. We also hypothesized that yellow model frogs would receive more attacks from predators than the more common red morph. Because perception of the frogs' warning signals is likely to differ among viewers (Maan and Cummings 2012), we also used visual models to ask whether the yellow and red morphs are distinct and conspicuous, as they are to human observers, under other relevant visual systems.

Methods

Mark-recapture

Study site

We conducted a capture-mark-recapture (CMR) study of *O. pumilio* in a 0.75 ha area of secondary-growth forest on the northwest tip of the island of Bastimentos (9.3468°N, 82.2064°W) in the Bocas del Toro Archipelago of Panama. Frogs in this population differ in dorsal color but all have white ventral coloration. Dorsal coloration falls into two groups (red and yellow) that can be reliably distinguished both statistically and by human observer and each morph is more or less evenly distributed across the study area (Richards-Zawacki et al. 2012). Because the frogs in this population each have a unique pattern of dorsal black patterning, we were able to use photographs to identify individuals. The utility of this method is supported by a previous study of this population where photo-based identifications were confirmed using microsatellite genotyping (0.3 % misidentification rate, Richards-Zawacki et al. 2012).

Sampling

Frogs in our study population are diurnal and active throughout the year. The entire study area was surveyed on 10 occasions between July 2007 and May 2012 (July 2007; March, June and October 2008; January, April and August 2009; June 2010, 2011; May 2012). During each survey period, frogs were captured by hand by a team of people (3–5) during one to three survey days within a 4 day period. The frogs were transported to the Smithsonian Tropical Research Institute's (STRI) Bocas del Toro Research Station where digital photographs of the dorsal and ventral surfaces, as well as body size (snout-vent

length, SVL) measurements of each frog were taken. Frogs were classified by sex and the tip of the fourth toe of each hind limb was removed for genetic analysis (see Richards-Zawacki et al. 2012). Adult frogs in this population range from 18 to 22 mm SVL with males (avg. 19.78 mm) being slightly smaller than females (avg. 20.23 mm). The sex of adult frogs was determined by the presence (male) or absence (female) of a dark throat patch. Because males usually exhibit dark throat patches by 18.00 mm SVL, frogs smaller than this were classified as juveniles (range = 11.0–17.9 mm) and larger frogs were classified as adults. Our previous work in this population found a 3.6 % error rate using this method of sex discrimination (Richards-Zawacki et al. 2012). While in captivity frogs were housed individually in small plastic containers with moist paper towel substrate. They were fed termites and fruit flies and misted daily. Frogs were released at their point of capture after the last frogs from that survey had been processed, always within 4 days of capture.

Modeling the effects of polymorphism on survival

We used open population Cormack-Jolly-Seber (CJS) models in program MARK (White and Burnham 1999) to estimate the apparent survival and detection rate of adult male and female *O. pumilio* and to test the hypothesis that these parameters differ among color morphs. These models assume that marked individuals in the population at time i have the same probability of survival to time $i + 1$ and the same probability of recapture at time $i + 1$. Because male, female and juvenile *O. pumilio* show different patterns of habitat use and movement that could violate this assumption (Pröhl and Berke 2001), we modeled each sex separately and only used the capture histories of adult frogs in model development. For each sex we tested 16 alternative models where survival and detection rate were either (i) constant, (ii) dependent on time, (iii) dependent on color morph, or (iv) dependent on both time and color morph.

We used the bootstrap goodness of fit test in MARK with 500 iterations to estimate the variance inflation factor (\hat{c}) and fit of the most general model to the data. Because our \hat{c} values tended to be greater than 1 (indicating overdispersion), model selection and ranking was based on Akaike's small-sample-size-corrected quasi-likelihood information criterion (QAICc) using the \hat{c} value estimated for the most general model (Anderson and Burnham 2002). Using this metric, the best model has a score of zero and models with Δ QAICc scores greater than two can be considered substantially less well supported than the best model. For nested models with Δ QAICc scores < 2 , the likelihood ratio test in MARK was used to test the null hypothesis that the reduced model is a better fit to the data than the model with additional parameters.

Predation experiment

In July 2012 we conducted a predation experiment at two field sites on the island of Bastimentos: (1) the polymorphic site of the CMR study (described above) and (2) La Loma (9.3179°N, 82.1547°W), a secondary growth forest site where frogs are monomorphic. Frogs from the La Loma population are bright orange/red dorsally, with variable numbers of small black spots, and white ventrally. Model frogs were constructed by pouring melted Van Aken modelling clay into silicone molds following Yeager et al. (2011). The clay models that resulted were similar in size (20 mm SVL) and shape to adult *O. pumilio*. We used red and yellow clay for our models, which we matched by eye to the

brightest yellows and reds from among our photographs of frogs from the CMR site. Black spots were drawn onto the clay using a Sharpie® marker.

Clay replica frogs were placed along transects in *O. pumilio* habitat at both sites. Models were placed in pairs (one red, one yellow) about 0.3 m apart perpendicular to the transect. A total of 400 replica pairs were placed at each site with 2 m spacing between them. Before placing the models on the ground, the area where they were to be placed (~30 cm diam.) was cleared of dead leaves and detritus to prevent visual obstruction of the models. Models were collected after 48 h and scored as either attacked or not attacked based on the presence or absence of impressions made in the soft clay (Brodie 1993; Saporito et al. 2007). Some of the attacks could be broken down further according to predator type based on the presence of impressions made by beaks (birds), large incisors (mammals), or claws (crabs), though a large number of marks were made by unknown assailants. Missing models were excluded from the analysis. Chi square of goodness of fit tests were used to assess whether coloration was a predictor of predation risk at each site and Chi square tests of independence were used to assess whether attack rates differed across predator types.

Visual modeling

Measurements of frog phenotype

The spectral reflectance of the dorsum of 10 males from La Loma and 10 males of each phenotype (red and yellow) from the CMR site were measured in June 2010 using a StellarNet EPP2000Cs UV–Vis spectrophotometer (StellarNet, Inc., Tampa, FL) and an R600-8 reflectance probe. The light source was a combination of a SL1 Tungsten Krypton lamp and a SL1-Blue UV-LED lamp. To avoid effects of lamp drift on reflectance measurements, a RS50 white standard was measured between frogs. The average of three dorsal reflectance measurements was calculated for each frog and used in subsequent analyses.

Measurement of background reflectance and irradiance

Spectral reflectances of leaves where *O. pumilio* were encountered (20 brown and 20 green leaves) were measured in June 2011 using an Ocean Optics Jaz portable spectrometer (Ocean Optics, Inc., Dunedin, FL) with an internal Jaz-PX pulsing xenon light source and a QR400-7-SR-BX reflection probe. A WS-1 white standard was measured between leaves. Ambient light measurements were also taken in *O. pumilio* habitat using the Ocean Optics Jaz unit with a QP400-2-UV–VIS fiber and a CC-3-UV-S cosine corrector. Downwelling absolute irradiance measurements were taken on a sunny morning in June 2011 from an open canopy (high light) habitat as well as a nearby closed canopy (low light) site during the peak period of *O. pumilio* activity. When switching from reflectance to irradiance measurements, calibration was accomplished using an Ocean Optics LS-1-CAL tungsten halogen lamp.

Modeling frog phenotypes under different visual systems

To estimate how each frog phenotype would be perceived by conspecifics and potential predators, we used visual models developed by Endler and Mielke (2005) and Vorobyev and Osorio (1998) as implemented in the program AVICOL (Gomez 2006). Inputs into the

Endler and Mielke (2005) model included (1) dorsal spectral reflectance measurements of male *O. pumilio*, (2) downwelling irradiance measurements from *O. pumilio* habitat (open and closed canopy), and (3) absorptance spectra of either the four cone classes of an avian visual system (Blue tit, *Parus caeruleus* from Hart and Vorobyev 2005), the three cone classes of *O. pumilio* (from Siddiqi et al. 2004, provided by T. Cronin), or the two cone classes of a crab visual system (fiddler crab, *Uca tangeri* from Jordão et al. 2007, provided by T. Cronin). For the Vorobyev and Osorio (1998) model, spectral reflectance measurements of leaf substrates (brown and green) were also used. For the Vorobyev and Osorio (1998) model, photoreceptor proportions (η_i) and Weber fractions (v_i) for both visual models were from Siddiqi et al. (2004). To ensure that our results are comparable with other studies of *O. pumilio* that have used this model (e.g., Siddiqi et al. 2004; Maan and Cummings 2012), we calculated photoreceptor noise (ω_i) as v_i/η_i and calculated deltaQ using ω for the long wavelength sensitive (LWS) cone in each visual system instead of using the default options in AVICOL.

The Endler and Mielke (2005) model was used to estimate spectral distances between individuals and morphs in tetrachromatic (avian) and trichromatic (*O. pumilio*) color space. Under this model, color space is represented by Cartesian coordinates in three-dimensions (x, y, z) for tetrachromatic visual systems and two-dimensions (x, y) for trichromatic visual systems (see Endler and Mielke 2005 for relationship between Cartesian coordinates and relative stimulation of cone classes). An analogous representation is not available for dichromatic (e.g., crab) color space.

The Vorobyev and Osorio (1998) model was used to estimate differences in conspicuousness among individuals and morphs in tetrachromatic (avian), trichromatic (*O. pumilio*) and dichromatic (crab) visual systems. In this model, a frog's contrast, or conspicuousness in a particular environment is measured in terms of chromatic (deltaS) and achromatic (deltaQ, also referred to as brightness or luminance) contrasts. These variables are measured in units called 'just noticeable differences' (jnds) where a value greater than 1 indicates that the difference (in this case between frog and substrate) is discernible by a viewer according to the properties of its visual system (Siddiqi et al. 2004).

Results

Capture-mark-recapture

While the relative frequencies of yellow and red morphs fluctuated somewhat, the 95 % confidence intervals on the frequency of the yellow morph overlaps for all 6 years of our study (Fig. 1a). This suggests that morph frequencies remained relatively stable over the study period. For our CJS modeling exercise, the bootstrap procedure did not indicate significant lack of fit of the most general model (survival and detection rates both time and color morph dependent) for males ($N = 645$, $\hat{c} = 3.978$, $p = 0.250$) or females ($N = 532$, $\hat{c} = 4.085$, $p = 0.432$). The best supported models ($\Delta\text{QAICc} < 2$) for both sexes included models in which both survival and detection rate were constant across time and among color morphs and models where survival or detection rate differed among color morphs (Table 1; see Table S1 in online supplementary material for summary of results for all 16 models). However, for both males and females, likelihood ratio tests indicated support for the reduced model (null hypothesis, survival and detection rates constant) over models where survival (Male: $\chi^2_1 = 0.343$, $p = 0.558$; Female: $\chi^2_1 = 1.219$, $p = 0.270$) or detection rate (Male: $\chi^2_1 = 0.08$, $p = 0.778$; Female: $\chi^2_1 = 0.357$, $p = 0.550$) differed

among morphs. Considering only the best-supported models that included variation in survival among color morphs (Males: $\Delta\text{QAICc} = 1.676$; Females: $\Delta\text{QAICc} = 0.803$), the 95 % confidence intervals on estimates of survival overlapped for both sexes and color morphs (Fig. 1b), although the difference between yellow females and yellow males appears to be approaching significance. Taken together, the CMR model analyses suggest that survival rates in this population do not differ significantly among sexes or color morphs and that morph frequencies have remained relatively stable over the course of our study.

Predation experiment

Of the 1,600 models placed across two sites, 202 (12.6 %) were attacked. This is similar to attack rates observed in other clay model studies of poison frogs (7.4–12.4 %; Saporito et al. 2007; Noonan and Comeault 2009; Chouteau and Angers 2011; Hegna et al. 2011, 2012). The frequency of attacks was higher at the CMR site (17.0 %) than at La Loma (8.3 %) ($\chi^2_1 = 23.56$, $p < 0.001$). The proportion of attacks made by different predator types differed among the two sites as well ($\chi^2_3 = 19.66$, $p < 0.001$) with the CMR site having more attacks attributed to crabs and La Loma having more attacks attributed to mammals (Fig. 2a). The rate of bird attacks was similar among sites (10.6 % at La Loma and 12.5 % at CMR site). Phenotype was not a significant predictor of predation at either site (CMR site: $\chi^2_1 = 0.36$, $p = 0.5485$; La Loma: $\chi^2_1 = 0.74$, $p = 0.3897$; Fig. 2b). The proportion of attacks made by different predator types also did not differ among red and yellow models at either site (CMR site: $\chi^2_3 = 4.39$, $p = 0.223$; La Loma: $\chi^2_3 = 1.59$, $p = 0.662$).

Visual modeling

Using the Endler and Mielke (2005) model, the yellow and red morphs from the CMR site fall out into different regions of color space in both a tetrachromatic bird visual system (Fig. 3a) and the trichromatic visual system of the frog (Fig. 3b). The color space occupied

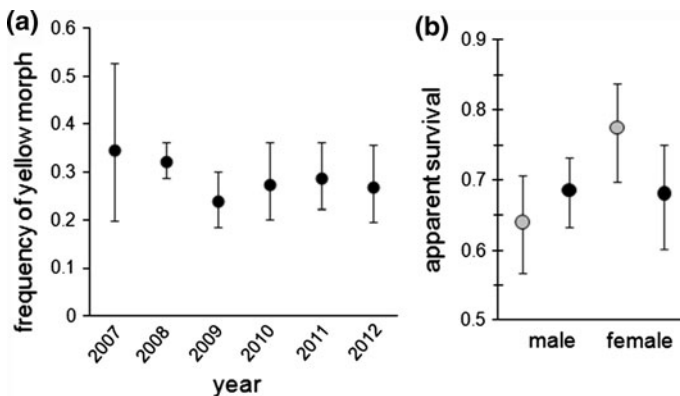


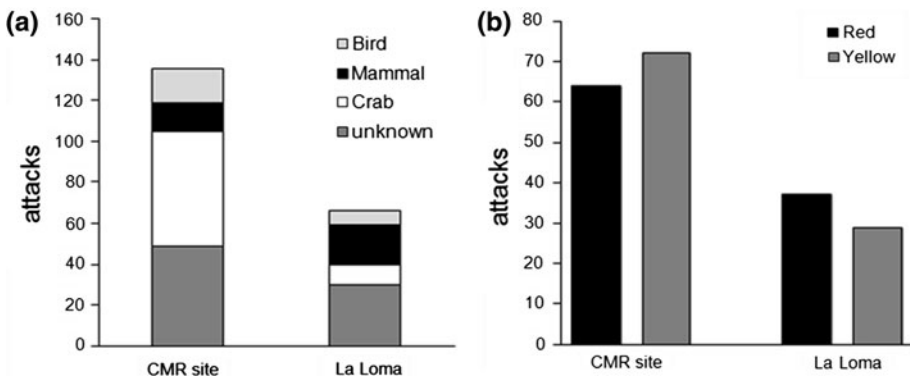
Fig. 1 Results from CMR study: **a** frequency of the yellow morph by year, and **b** apparent survival of yellow and red morphs as estimated by CMR models. For each sex the estimated survival and 95 % confidence intervals are shown for the CJS model where survival is dependent upon color and detection rate is constant. *Gray circles* denote yellow frogs and *black circles* denote red frogs

Table 1 Summary results of CJS model ranking

Model structure		QAICc	Δ QAICc	W	K	QDeviance
Survival	Detection					
<i>Males</i>						
Constant	Constant	255.155	0	0.455	2	49.208
Color	Constant	256.831	1.676	0.197	3	48.865
Constant	Color	257.094	1.939	0.173	3	49.129
...
Color \times time	Color \times time	291.019	35.864	<0.001	29	28.261
<i>Females</i>						
Constant	Constant	172.323	0	0.422	2	37.685
Color	Constant	173.126	0.803	0.282	3	36.465
Constant	Color	173.989	1.666	0.183	3	37.328
...	...	217.849
Color \times time	Color \times time		45.526	<0.001	31	21.266

The best three models (Δ QAICc < 2) and the most general (starting) models for each sex are shown

Model selection based on $\hat{c} = 3.978$ for males and 4.085 for females. Table labels are AIC score corrected for small sample size (QAICc), difference in QAICc between current and best model (Δ QAICc), QAICc weights (W), number of parameters in the model (K) and model deviance (QDeviance). “...” indicates models not displayed

**Fig. 2** Number of attacks on clay models by **a** predator type and **b** model color across two sites

by the red/orange phenotype from the La Loma population overlaps with that of red frogs from the CMR site under both visual systems. Models using open versus closed canopy light spectra produced nearly identical results (see Fig. S1 in online supplementary material).

Results from the Vorobyev and Osorio (1998) model suggest that all three morphs would appear highly conspicuous (ΔS and $\Delta Q \gg 1$) under an avian visual system (Fig. 3c), the *O. pumilio* visual system (Fig. 3d) and a crab visual system (Fig. 3e). Yellow frogs from the CMR site tend to have lower chromatic contrasts (ΔS) to leaf substrates but higher achromatic contrasts (ΔQ) than the red morph they are sympatric with. Frogs from La Loma tend to be intermediate in chromatic contrast but as high as or higher than

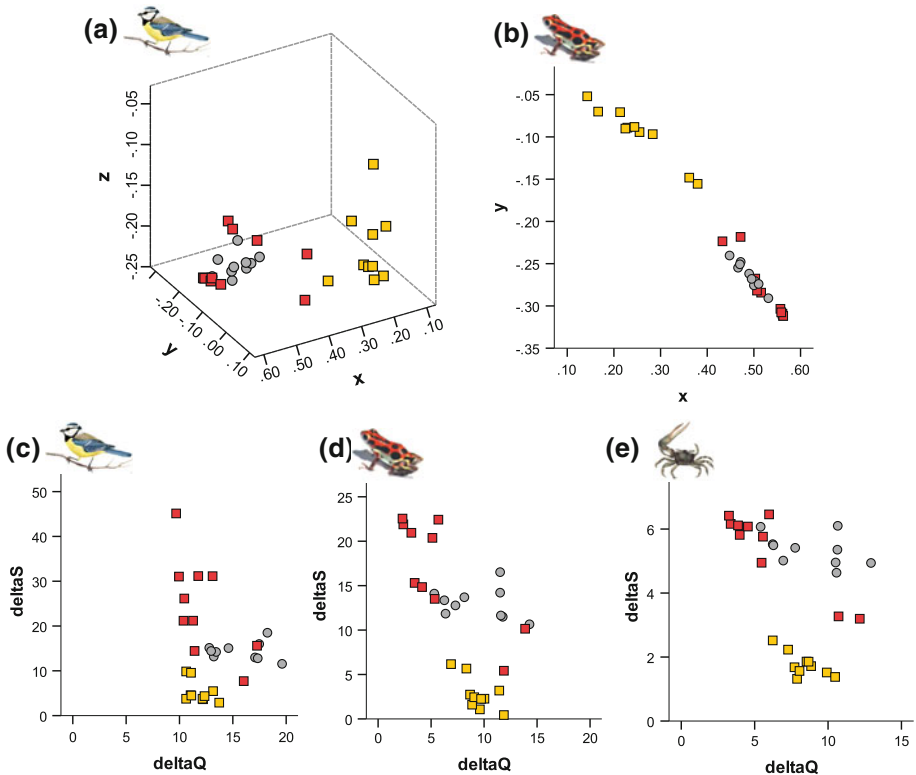


Fig. 3 Results of visual modeling of the phenotypes of *O. pumilio* morphs. Dorsal coloration of 10 males from the CMR site (squares: red = red morph, yellow = yellow morph) and 10 males from La Loma (gray circles) under open canopy lighting are plotted using the Endler and Mielke (2005) model in the color space of **a** a tetrachromatic bird (*P. caeruleus*) visual system and **b** the trichromatic *O. pumilio* visual system. Measures of the chromatic (deltaS) and achromatic (deltaQ) contrast (measured in jnds) of these same frogs using the Vorobyev and Osorio (1998) model are also shown for **c** *P. caeruleus*, **d** *O. pumilio*, and **e** *U. tangeri* visual systems. For **c–e**, open canopy lighting and a green leaf background was used

yellow CMR frogs in achromatic contrast. These trends are seen in all three visual systems and regardless of which background (brown or green leaf) or lighting environment (open or closed canopy) was used (see Fig. S2 in online supplementary material).

Discussion

The results of our visual modeling exercise suggest that the red and yellow *O. pumilio* morphs from the polymorphic population on Bastimentos are separated in color space and contrast highly with natural background substrates under a variety of forest lighting conditions. This holds true whether the observer is human (Richards-Zawacki et al. 2012), avian, crab, or another frog. Thus, both morphs possess warning coloration that is distinct yet conspicuous across a variety of visual systems and are similarly unpalatable. In this situation aposematism theory would predict predators to exert stabilizing selection resulting in the eventual loss of the less-common morph (Ruxton et al. 2004). The tendency

for the more common red frogs in this population to mate assortatively by color also suggests that yellow should decrease in frequency over time. However, we found no evidence that this is occurring in our study population.

Over our 6-year CMR study, the frequency of red and yellow fluctuated somewhat from year to year but showed no discernible trend. Red frogs were consistently about twice as common as yellow frogs. If predators are indeed learning to associate color or other spectral properties with unprofitability by attempting to eat these frogs, that learning process should be faster for red frogs giving them a selective advantage over the less common yellow morph (Ruxton et al. 2004; Mappes et al. 2005). However, the best supported CJS models suggest that survival does not differ between morphs.

Similarly, our clay model study found no difference in the number of attacks on red versus yellow. This was true even in the La Loma population where the yellow morph does not naturally occur and, judging by the organisms that attacked the models, the make-up of the predator community is different from that of the polymorphic population. These results contrast with studies of other poison frog species where local or more common morphs received fewer attacks than foreign or rare forms (Noonan and Comeault 2009; Chouteau and Angers 2011).

Unlike previous poison frog clay model studies, we treated attacks by crabs, which were common (41 % at CMR site, 15 % at La Loma) and easily distinguishable in our dataset, as distinct from other “unknown” predators. The commonness of crab attacks in our dataset could reflect a real difference between the predator community on Bastimentos and previous clay model study sites. While the extent to which crabs use visual cues in foraging is not clear, color signals are sometimes used in crab social signaling (Hemmi et al. 2006; Cummings et al. 2008) and our visual modeling study suggests that yellow and red *O. pumilio* appear both conspicuous and distinct in a crab visual system. Crab predation on poison frogs has been documented (Gray and Christy 2000) and we have observed crabs attacking and chasing *O. pumilio* on Bastimentos (C. Richards-Zawacki, unpublished). For these reasons, we have not ruled out the possibility that predation by crabs, as well as by more traditional visual predators such as birds, could be important in shaping the evolutionary trajectory of the Bastimentos *O. pumilio* polymorphism.

Taken together, our analyses provide no clear evidence for differential predation or survival among sympatric red and yellow *O. pumilio* color morphs. The frequency of the less common yellow morph fluctuated somewhat but showed no clear downward trend during the 6 year timeframe of our study. While this suggests the potential for stability, longer term studies are needed to provide a clearer picture of the evolutionary dynamics of this color polymorphism.

One explanation for the persistence of polymorphism on Bastimentos might be a relaxation of selection due to lower levels of predation (Chouteau and Angers 2012). A similar argument has been proposed to explain the greater diversity of color morphs on the islands of the Bocas del Toro archipelago in comparison with mainland regions (Hegna et al. 2012), and faster diversification in aposematic taxa in comparison to non-aposematic sister taxa (Przecek et al. 2008). However, results from our clay model experiment fail to provide support for this hypothesis as the attack rate was twice as high (17.0 %) in the polymorphic region of Bastimentos as it was in the monomorphic La Loma population (8.3 %). Clay model studies conducted in mainland populations of *O. pumilio* have yielded attack rates between these two estimates (Hegna et al. 2011, leaf litter background only = 11.2 %; Saporito et al. 2007, red phenotype on leaf litter only = 11.0 %) suggesting that predation rates are not necessarily lower for island populations. It is also possible, especially when considering the increasing impact of human land use on the landscape of Bocas

del Toro, that the current predator community is different from that under which the polymorphism originally arose. If so, we might not detect differences in predation among morphs or populations even if they were important in shaping diversity we see in this species today.

Another explanation for persistence of the Bastimentos polymorphism might be that despite being distinguishable and conspicuous, both red and yellow warning signals are equally effective in deterring predators. Results of a clay model study by Hegna et al. (2012) support the idea that red coloration is a more effective warning signal than green in *O. pumilio*, regardless of which signal is local. Perhaps yellow is equally as effective as red? Both red and yellow are ‘typical’ warning colors found in association with anti-predator defenses across the animal kingdom (Mappes and Alatalo 1997). While empirical studies have largely been limited to bird predators, support for both innate avoidance of typical warning colors (Smith 1975; Schuler and Roper 1992; Marples et al. 1998) and avoidance of those colors learned by observing the behavior of conspecifics (Skelhorn 2011) has been found. If predators often generalize (Mappes and Alatalo 1997; Ruxton et al. 2008), considering both red and yellow morphs of *O. pumilio* to be highly unprofitable, the polymorphism could remain stable. The polymorphism could also be stabilized if certain predators avoid red frogs while others avoid yellow, leading to similar rates of predation across morphs. While this sort of differential predation was not observed for bird, crab or mammal predator classes in our clay model study, it is possible that such a pattern existed within the approximately 40 % of attacks made by unknown predators.

Behavioral differences among morphs could also affect the persistence of this polymorphism. Differences in the microhabitats used by *O. pumilio* during courtship (Pröhl and Ostrowski 2011; Rudh et al. 2011), and in foraging and escape behavior (Pröhl and Ostrowski 2011), support the idea that courtship and anti-predator behavioral strategies have diverged among allopatric populations. If similar behavioral differences have evolved among sympatric morphs this could help explain the persistence of polymorphism on Bastimentos. Color-assortative mating preferences could also affect the stability of the polymorphism (Richards-Zawacki and Cummings 2011). Because the red morph is more common, appears to be dominantly inherited, and shows a stronger tendency toward assortative mating in the wild (Richards-Zawacki et al. 2012), the yellow morph might be expected to disappear from the population over time. However, if mating preferences are learned rather than innate, modeling studies suggest that this sort of polymorphism can be stable (Kalmus and Maynard Smith 1966; Seiger 1967) and even lead to sympatric speciation (Verzijden et al. 2005).

Whether the Bastimentos polymorphism remains stable over longer periods of time will likely depend upon the balance between natural selection, sexual selection (Richards-Zawacki and Cummings 2011) and genetic drift (Tazzyman and Iwasa 2010; Chouteau and Angers 2012) among color morphs. Considering the many factors at play, the evolutionary trajectory might be quite different in other polymorphic populations, especially where sympatric forms differ dramatically in conspicuousness and not all morphs possess typical warning coloration. Additional studies of the efficacy of alternative color signals are needed to clarify the relative roles of predation and other selective forces in the evolution of aposomatic variation in poison frogs and other polytypic species.

Acknowledgments We thank Narissa Bax, Sebastian Castillo, Ricardo Cossio, Anisha Devar, Deyvis Gonzalez, Eli Hornstein, Danny Lenger, Tom Richards, Becky Richards, Eric Rightley, Tracy Stetzinger, Geoff Zawacki and Amanda Zellmer for help with the mark-recapture study and to Anisha Devar for help with the predation experiment. The STRI provided logistical support for this project and we are particularly

grateful to Gabriel Jacome and Plinio Gondola of the Bocas del Toro Field Station. This study was financially supported by short-term fellowships from STRI to CLRZ and JY, postdoctoral fellowships from the National Science Foundation (Award No. 0801165) and the University of California President's office to CLRZ, and a Tulane Dean's grant to HPSB. The Panamanian National Authority for the Environment (ANAM) provided research permission for this study. This work complied with IACUC protocols (University of Michigan No. 09765, Tulane University No. 0832 and STRI No. 2007-17-12-15-07).

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