



Colour and Escape Behaviour in Polymorphic Populations of an Aposematic Poison Frog

Matthew B. Dugas*, Susannah R. Halbrook*, Allison M. Killius*, Jillian F. del Sol† & Corinne L. Richards-Zawacki*

* Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA

† Biology Department, Hendrix College, Conway, AR, USA

Correspondence

Matthew B. Dugas, Department of Biology,
Case Western Reserve University, DeGrace
Hall, 2080 Adelbert Rd., Cleveland, OH
44106, USA.

E-mail: matthew.b.dugas@gmail.com

Received: February 13, 2015

Initial acceptance: April 14, 2015

Final acceptance: April 19, 2015

(W. Koenig)

doi: 10.1111/eth.12396

Keywords: aposematism, Dendrobatidae,
flight initiation distance, integrated
phenotype

Abstract

The phenomenon of aposematism, or the pairing of antipredator defence with conspicuous or distinctive signals, serves as an excellent example of how traits act in concert to shape fitness. Not only does this complex phenotype require the integration of multiple traits, it alters the fitness pay-offs of yet others. The protection offered by aposematism may, for example, reduce the costs associated with foraging or sexual display. Thus, well-protected aposematic lineages should be bolder, more active and less likely to respond to perceived threats of predation than more cryptic lineages. Comparisons of differently coloured morphs of the polytypic strawberry poison frog (*Oophaga pumilio*) have supported the predicted behavioural correlates of aposematism, with the exception of those regarding responsiveness to simulated predators. We tested the key prediction that aposematic coloration will be associated with reduced sensitivity to predators in two polymorphic *O. pumilio* populations. The novel approach of studying polymorphic populations allowed us to assess the effect of colour in the absence of potentially confounding habitat differences. We found that colour was associated with the probability that a frog would attempt escape and the distance at which it fled, but only in one population, and not in the predicted direction. An overall comparison of the two populations superficially supported our predictions, but this pattern actually arose because frogs occupying higher perches were less likely to respond, a pattern that may reflect the value of high perches and the costs associated with returning to them after attempted escape. These results highlight the complexity of the relationship between predators and prey, the challenges associated with understanding how and why traits are correlated, and the intimate ties between behaviour and morphological evolution.

Introduction

Traits often act in concert to shape the fitness of individuals, with the costs and benefits of one trait depending critically on the expression of others (Murren 2012). The phenotypic integration that can arise from such functional and evolutionary relationships is well illustrated by the phenomenon of aposematism, the pairing of antipredator defences with signals that advertise unprofitability (Ruxton et al. 2004). The

benefits of defence are enhanced by signals because signals reduce the frequency and costliness of predator encounters, while in the absence of defence, signals drawing the attention of predators would be quite costly (Ruxton et al. 2004). Both defences and signals may in turn be built from a large suite of physiological and behavioural traits that underlie their expression (Mochida 2009; Santos & Cannatella 2011). Divergent antipredator strategies such as aposematism and crypsis not only require such

integration of physiology, morphology and behaviour, they also alter the way selection acts on yet other suites of traits (Stankowich & Blumstein 2005).

By reducing the frequency of costly encounters with predators, the protection that aposematism confers can lower the costs of otherwise risky behaviours, including foraging and sexual display (Speed et al. 2010). Conspicuous behaviours should then be advantageous to a greater extent in aposematic lineages than in cryptic ones and thus performed more often. Efforts to escape potential predators might, on the other hand, be more costly in aposematic than cryptic lineages: allowing predators to evaluate warning signals may reduce the risk of attack (Cooper et al. 2009a), and a higher proportion of encounters are likely to be costly false alarms for well-protected individuals (Cooper 2003). Exceptional opportunities to test these predictions come from systems in which closely related lineages differ in the antipredator strategies they pursue (Cooper 2006; Mochida 2009).

Poison frogs (Dendrobatidae) vary extensively in coloration and toxicity, with numerous independent transitions (in both directions) between crypsis and aposematism, and broad comparative studies of this group have supported the hypothesis that traits underlying aposematism evolve together (Santos & Cannatella 2011). The behavioural correlates of putatively aposematic or cryptic coloration are less broadly studied, but have received some attention in the polytypic strawberry poison frog (*Oophaga pumilio*). While this frog is phenotypically diverse throughout its range, this variation is extraordinary in and around the Bocas del Toro archipelago of Panama, where recently isolated populations display colour variation spanning nearly the entire visible spectrum (Rudh et al. 2007). Comparisons among allopatric Bocas del Toro *O. pumilio* populations broadly classified as conspicuous and cryptic indicate that males of conspicuous morphs display in more exposed sites and are more active (Pröhl & Ostrowski 2011; Rudh et al. 2011) and are perhaps more aggressive and explorative (Rudh et al. 2013). Similar patterns are also suggested by work in a polytypic congener (*O. granulifera*: Willink et al. 2013). Contrary to predictions, however, a comparison of escape behaviour between a visually conspicuous and an inconspicuous *O. pumilio* morph suggests that conspicuous males are more sensitive to predators (Pröhl & Ostrowski 2011). This latter result is problematic, as reduced sensitivity to predators is one of the most clearly predicted behavioural correlates of aposematism (Ruxton et al. 2004; Cooper et al. 2009b).

We built on previous work by testing the key prediction that conspicuous coloration will be associated with reduced responsiveness to predators (Stankowich & Blumstein 2005) in two polymorphic populations of *O. pumilio*. While comparisons of allopatric populations are often all that is possible, they can be limited by confounding differences among studied habitats (Cooper & Avalos 2010). The expression of fear can be shaped on both ecological and evolutionary time scales by innumerable factors entirely independent of prey coloration or chemical defence, including the physical properties of the habitat, the identity and density of predators, or individuals' prior experience with them (Lima & Dill 1990; Stankowich & Blumstein 2005). In polymorphic populations, these confounds are largely absent. We compared one aspect of display site selection (perch height) and two components of escape behaviour (probability of escape attempt and flight initiation distance) in calling males from two polymorphic populations of *O. pumilio* in Panama: (1) Isla Bastimentos and (2) Dolphin Bay, on the mainland (Fig. 1). Following previous work, we compared these two populations, predicting that frogs from the overall more conspicuous population (Bastimentos – see Methods) would call from more risky perch sites (higher perches, given that upward escape is not practical for slow-moving frogs: Cooper et al. 2009a,b) and would be less sensitive to simulated predator encounters. We tested similar predictions within each polymorphic population, considering these an even more powerful test for the hypothesized relationship between colour and behaviour because all individuals in each polymorphic site shared a common habitat.

Methods

Study Species and Study Sites

We studied polymorphic populations of *O. pumilio* at two study sites: Isla Bastimentos and Dolphin Bay. The Isla Bastimentos study site is a 0.75 ha area of mixed secondary forest and early successional growth, located on the north-west tip of Isla Bastimentos (9.3468°N, 82.2064°W). In this population, *O. pumilio* have brown/black dorsal patterning with red, yellow and sometimes greenish dorsal background colour (Fig. 1; Maan & Cummings 2008). How this polymorphism arose remains unclear; translocation by humans (Rudh et al. 2007) or a recent (~5200 ya: Anderson & Handley 2002) dispersal corridor between present day Isla Bastimentos (where most frogs are red) and Isla Colon (where frogs are yellow/green)



Fig. 1: Representative photographs illustrating *O. pumilio* dorsal colour variation along two principal component axes, calculated independently for each population. Individuals from Isla Bastimentos (a, b) and Dolphin Bay (c, d) are presented left to right, in increasing scores on PC1 (a, c) and PC2 (b, d).

seem reasonable hypotheses for the presence of yellow dorsal coloration at this site. The Dolphin Bay study site is a ~40 ha area of mixed secondary forest and cacao on the north end of the Aguacate Peninsula of mainland Panama (9.2210°N, 82.2185°W). Here, black dorsal patterning is absent, and red (with blue legs) and blue *O. pumilio* occur alongside intermediately coloured individuals (Fig. 1). This polymorphism occurs in what appears to be a transition zone between red frogs with blue legs, which is the most common phenotype on the adjacent island of San Cristobal and north of the transition zone on the mainland, and entirely blue frogs, which are found further south on the Aguacate Peninsula (Summers et al. 2003).

Overall, Bastimentos frogs are considerably more conspicuous than Dolphin Bay frogs (Fig. S1). Within Bastimentos, red morphs are more visually conspicuous than yellow morphs (Fig. S1, Richards-Zawacki et al. 2013). Blue Dolphin Bay frogs are often considered the least conspicuous of all *O. pumilio* morphs, while populations similar to the Dolphin Bay red morph have been classified as either conspicuous or moderately conspicuous in previous studies (Rudh et al. 2011; Maan & Cummings 2012). Overall, frogs from Bastimentos are more toxic than those from Dolphin Bay, but both are among the more toxic *O. pumilio* populations (Maan & Cummings 2012). Differences in chemical defence among morphs within populations seem plausible, although local

variation in alkaloid-rich prey abundance might make morphs similar at each site (Saporito et al. 2007a).

Simulated Predator Approaches

We simulated predator approaches in May–July 2014, between 700 and 1200 h, when *O. pumilio* activity is highest (Graves 1999). We used only calling males in this experiment, as they could be easily located without disturbance and they were engaged in a maximally conspicuous activity that would be costly to abandon unnecessarily. We also avoided sampling males engaged in courtship. We searched by walking slowly and systematically through sites. Upon visually locating a calling male, we slowly moved (if necessary) to a position ~3 m from the frog (Pröhl & Ostrowski 2011). Although we made sure we were within the frog's range of vision, attempts to standardize approach angle proved ineffective as (1) we had to approach in a straight line and often had limited paths clear of obstruction and (2) the frog often changed orientation between calling bouts.

After moving into position, we waited for the male to resume calling and then approached him at an agreed-upon and practiced pace (~1 m/sec); we chose a relatively slow pace because frogs are more likely to respond to slow approach (Cooper et al. 2009b). If and when the frog left a perch, we dropped a marker at our position when it fled, and then pursued the frog. We then measured flight initiation distance (predator–prey distance when prey moves: Stankowich & Blumstein 2005) as the distance between the dropped marker and the perch; if we captured the frog by hand on the perch, we scored flight initiation distance as 0. We also measured the height of the perch; we measured all distances to the nearest cm using a flexible measuring tape. We then photographed the frog against a grey colour standard (details below) and removed the tip of one toe to identify observed males and avoid resampling the same individuals.

Colour Quantification

We hand-captured *O. pumilio* and took photographs of the dorsum in the field with a digital camera (Panasonic DMC-TS5, Kadoma, Osaka, Japan). We photographed each individual without a flash against a waterproof 18% grey standard (DGK Color Tools) and manually set white balance for each photograph. To score colour, we used the RGB Measure Plus plugin (Prodanov 2005) for ImageJ (Rashband 1997–2004) to obtain average red (R), green (G) and blue (B)

scores for 20 × 20 pixel areas of background coloration in each of five locations on the frog's body (Fig. 2). We did not sample colour from any region in which dirt, glare or shadow obscured colour and also avoided scoring colour from black dorsal spots within body regions (Fig. 2), as these are consistently coloured among morphs and do not mediate predation risk (Hegna et al. 2011). To permit standardization across photographs, we also recorded, for each, the average R, G and B values for a 20 × 20 pixel area of the grey standard background adjacent to the frog.

Within-photograph repeatability (Lessells & Boag 1987) of colour measurements was high (Table 1), and so we used means for further analyses. Because we photographed frogs in the field under non-standard conditions, we corrected mean colour scores by taking residuals of mean R, G or B frog colour regressed on R, G or B scores from the grey standard in each photograph (Loiseau et al. 2008). We then used a principal components analysis (PCA) to reduce the number of colour parameters for further analysis (Loiseau et al. 2008). While this approach allowed us to treat colour as a continuous variable, most authors have considered these populations to be primarily composed of discrete 'morphs' (Maan & Cummings 2008; Richards-Zawacki et al. 2013). Treating colour as continuous accommodates the possibilities that (1)

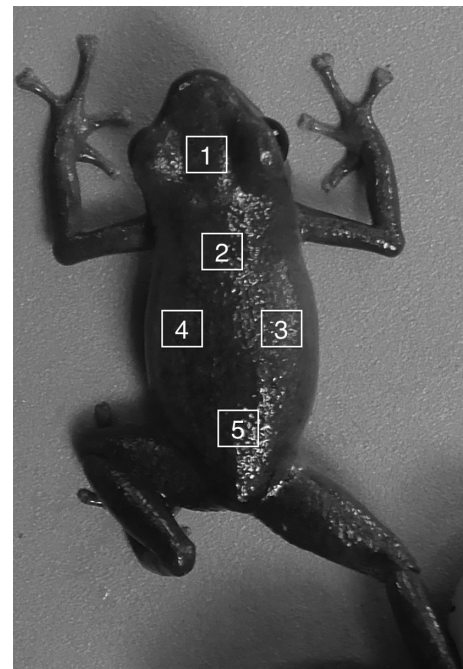


Fig. 2: Locations of five body regions from which we sampled dorsal coloration of male *O. pumilio*.

Table 1: Repeatability (*r*) of red (R), green (G) and blue (B) colour scores from photographs of *O. pumilio* males from two polymorphic populations

Population	Colour value	<i>F</i>	df	<i>p</i>	<i>r</i>
Bastimentos	R	6.0	41,144	<0.001	0.77
	G	19.4	41,144	<0.001	0.94
	B	6.0	41,144	<0.001	0.77
Dolphin Bay	R	10.5	66,225	<0.001	0.80
	G	10.0	66,225	<0.001	0.79
	B	10.8	66,225	<0.001	0.80

observer defined ‘morphs’ are not meaningful biological categories (Murren 2012) and (2) admixture among lineages produces intermediate phenotypes. Nonetheless, we compared these continuous colour scores among ‘morphs’ (as categorized by eye by MBD, the most experienced author) to confirm that our colour scoring could be compared to previous work.

Statistical Analysis

We began by comparing Bastimentos and Dolphin Bay populations, considering one proxy for perch site riskiness: perch height, and two proxies for escape behaviour: (1) whether the frog attempted escape (i.e. moved from the perch) prior to capture and (2) flight initiation distance (FID). We compared perch height between populations using an independent samples unpaired *t*-test. For both metrics of escape behaviour, we were interested in including the covariate of perch height, as this could influence the frog’s ability to detect approaches and/or its perception of threat. We first used a logistic regression to compare attempted escape (y/n) between populations, including population, perch height and population \times perch height as fixed effects. We then used identical structure in a general linear model in which we entered FID as the response variable; while FID was not normally distributed (because of a high proportion of 0s – see Results), residuals from this model were. We took a similar analytical approach for within-population comparisons, replacing the term ‘population’ with continuous colour variables (PC1, PC2). We assessed the relationship between colour and either perch height or FID using general linear models and used a logistic regression to assess the relationship between colour and escape attempt (y/n) probability. Residuals for models of FID from both populations were normally distributed. Although we made directional predictions, we used two-tailed tests because of previous work

revealing patterns in the opposite direction (Pröhl & Ostrowski 2011). We used SPSS (v19) IBM Corp., Armonk, NY for PCA and SAS (v9.2) SAS Inc., Cary, NC, USA for all other analyses.

Ethical Note

These methods were approved by the Institutional Animal Care and Use Committees at both Tulane University (Protocol No. 0382R) and the Smithsonian Tropical Research Institute (Protocol No. 2014-0515-2017). Toe clipping is an established method of marking amphibians that causes little stress to individuals (Perry et al. 2011), and we always clipped a small toe on a hind foot to minimize any influence on the frogs’ locomotor ability. After capture and toe clipping, we released animals where they had been seen calling; previous work indicates that toe-clipped males quickly return to their territories, even when released up to 20 m away (McVey et al. 1981).

Results

Within-Population Colour Variation

We approached a total of 41 Bastimentos males; we categorized 28 of these as ‘red’ and 13 as ‘yellow’ by eye (Richards-Zawacki et al. 2013). In Dolphin Bay, we approached a total of 67 males; by eye, we categorized 19 as ‘blue’, 19 as ‘red’, and the other 29 as ‘intermediate’. For both the Bastimentos and Dolphin Bay populations, a PCA on residual R, G and B colour scores from photographs resulted in the extraction of two principal components (PCs); in both populations, these explained ~60% and ~35% of the variance, respectively (Table S1). Loadings were remarkably similar in both populations, with the first PC (PC1) associated with high G and B values, and the second component (PC2) associated with high R values (Fig 1, Table S1). Red and yellow Bastimentos morphs differed significantly in PC1 (*t*-test: $t_{39} = -10.5$, $p < 0.001$), but not in PC2 ($t_{39} = 0.06$, $p = 0.95$; Fig. S1). The three Dolphin Bay morphs differed significantly in both PC1 (GLM: $F_{2,64} = 16.5$, $p < 0.001$) and PC2 ($F_{2,64} = 19.9$, $p < 0.001$). Tukey *post hoc* comparisons revealed that on PC1, blue morphs differed from red and intermediate morphs ($p < 0.001$), but red and intermediate morphs were not significantly different from each other ($p = 0.12$). On PC2, red, blue and intermediate morphs were all significantly different from one another (all $p < 0.005$).

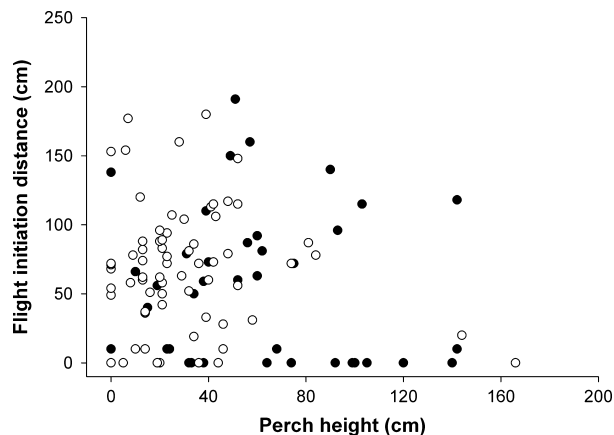


Fig. 3: Relationship between perch height and flight initiation distance in *O. pumilio* males from Isla Bastimentos (filled circles) and Dolphin Bay (open circles). A flight initiation of 0 indicates that the frog did not attempt escape and was captured by hand on the perch.

Perch Height and Escape Behaviour

The height of perches used by Bastimentos (60 ± 40 cm) and Dolphin Bay (31 ± 29 cm) males was significantly different ($t_{66,1} = 4.09$, $p < 0.001$; Fig 3). A smaller proportion of males attempted escape in Bastimentos (29/41) than in Dolphin Bay (60/67). While this difference was significant on its own (chi-square = 6.2, $df = 1$, $p = 0.013$), including the covariate of perch height in a logistic regression revealed that this difference in escape attempt probability was attributable to perch height (Wald chi-square = 5.53, $df = 1$, $p = 0.019$; Fig. 3), not population (Wald chi-square < 0.01, $df = 1$, $p = 0.95$). Although the effect of perch height was significant in Bastimentos only, the interaction between perch height and population was non-significant (Wald chi-square = 0.77, $df = 1$, $p = 0.38$; Fig 3). Flight initiation distance did not differ between the two populations ($F_{1,104} = 0.43$, $p = 0.51$) and was not predicted by perch height ($F_{1,104} = 1.81$, $p = 0.18$) or an interaction between these effects ($F_{1,104} = 0.02$, $p = 0.92$; Fig 3).

In Bastimentos, perch height was significantly and negatively associated with colour PC1 ($\beta \pm SE = -12.4 \pm 6.1$, $F_{1,38} = 4.15$, $p = 0.049$), but was not associated with colour PC2 ($F_{1,38} = 1.31$, $p = 0.26$). In tests of escape behaviour, interactions between colour and perch height were never significant (all $p > 0.59$), and so we present these results in Supporting Information (Table S2) and in text, present results of models with this effect removed. For Bastimentos males, both the probability that an individual would attempt escape and flight initiation distance were negatively

and significantly associated with colour PC1 and perch height (Table 2; Fig. 4), but not with colour PC2 (Table 2; Fig. 4). In summary, more blue and green (i.e. yellower: Fig. 1) Bastimentos males occupied lower perches, were less likely to attempt escape and waited until simulated predators were closer before fleeing; independently of colour, males calling from higher perches were less responsive to an approaching human.

In Dolphin Bay, perch height was significantly and positively associated with colour PC2 (PC1: $F_{1,64} = 1.06$, $p = 0.307$; PC2: $F_{1,64} = 7.2$, $p = 0.009$). However, this pattern was driven by two extreme observations, and after they were removed, this relationship disappeared (PC1: $F_{1,62} = 1.91$, $p = 0.17$; PC2: $F_{1,62} = 0.15$, $p = 0.70$). These two observations similarly drove patterns in an analysis of escape attempt probability (Appendix S2). Without including these two extreme observations in the analysis, interactions were non-significant (all $p > 0.19$), and so we again present these results in the Supporting Information (Appendix S2) and report results of tests without interactions in text (Table 2). Neither colour PC1 nor PC2 predicted the probability of attempting escape or flight initiation distance, and perch height was not significantly associated with escape behaviour in Dolphin Bay (Table 2, Fig. 4).

Discussion

Aposematic defence against predation is predicted to reduce the costs of behaviours otherwise associated with the risk of costly predator encounters, and thus to drive widespread behavioural differences among lineages pursuing divergent antipredator strategies (Speed et al. 2010). We found no support for the key prediction that visually conspicuous, putatively aposematic, *O. pumilio* males would be less responsive to simulated predator attacks than would less conspicuous individuals. In the red–yellow polymorphic Bastimentos population, more conspicuous red males, as predicted, occupied higher perches, but were more sensitive to a simulated predator approach. In the red–blue polymorphic Dolphin Bay population, we found no relationship between colour and behaviour. Our comparison of the two populations highlights the importance of considering alternative explanations for behavioural differences among allopatric lineages: Bastimentos males were less likely to attempt escape than were the less conspicuous Dolphin Bay males, but this pattern arose because Bastimentos males used higher perches and males on higher perches were less likely to attempt escape. This pattern can be explained

Table 2: Predictors of escape behaviour of male *O. pumilio* from two polymorphic populations (Bastimentos, Dolphin Bay)

Escape	Bastimentos					Dolphin Bay				
	Estimate	SE	Wald chi-square	df	p	Estimate	SE	Wald chi-square	df	p
attempt (y/n)										
Intercept	3.9	1.28				2.21	0.82			
Colour PC1	−1.26	0.54	5.42	1	0.020	−0.52	0.49	1.13	1	0.29
Colour PC2	−0.77	0.5	2.37	1	0.12	0.93	0.59	2.51	1	0.11
Perch height	−0.04	0.01	7.32	1	0.007	0.02	0.03	0.66	1	0.42
Flight initiation distance										
Intercept	1.78	0.23				66.91	9.56			
Colour PC1	−0.34	0.13	6.83	1,37	0.013	1.65	5.58	0.09	1,61	0.77
Colour PC2	−0.14	0.13	1.19	1,37	0.28	−1.72	5.98	0.08	1,61	0.78
Perch height	−0.01	0.003	7.36	1,37	0.010	0.15	0.29	0.27	1,61	0.60

We present estimates and standard error (SE) as well as test statistics and p-values for logistic regressions predicting the probability of attempted escape (y/n) and the distance at which frogs initiated escape (flight initiation distance; cm). Interaction effects were non-significant for all four models and are presented in supplemental material.

without evoking coloration, toxicity or aposematism by considering the costs associated with fleeing high perches: (1) high perches are valuable because they are associated with male mating success (Pröhl & Hödl 1999) and (2) poison frogs are slow and escape predators by jumping down to the leaf litter, so returning to high perches would take more time and energy than returning to low ones. While a similar study of two other *O. pumilio* populations did not directly test for a relationship between perch height and escape behaviour, the population that used higher perches in that study also responded less to simulated predators (Pröhl & Ostrowski 2011).

Overall, evidence thus far does suggest that conspicuous coloration in polytypic poison frogs is associated with reduced investment in avoiding potential predators (Rudh et al. 2011, 2013; Willink et al. 2013), with the notable exception of escape behaviour (Pröhl & Ostrowski 2011; this paper). Left unanswered are questions about the consequences and causes of these relationships. For example, if antipredator strategies and other behaviours are integrated via genetic mechanisms, intermorph matings in polymorphic populations could carry the cost of maladaptive combinations of coloration, toxicity and behaviour, thus contributing to the evolution of reproductive isolation among morphs (Rudh et al. 2011). On the other hand, if these associations arise primarily via plastic behavioural responses to experiences with predators (Lima & Dill 1990), not only will this constraint be absent, but novel colours of admixed offspring may allow the exploitation of new

space along the morphological and behavioural axes from aposematism to crypsis. Behavioural patterns that are plastic and/or sensitive to habitat constraints further highlight ambiguity about whether antipredator strategies constrain behaviours such as foraging and sexual display, or whether the importance of these behaviours instead drives the evolution of conspicuous coloration in toxic animals (Speed & Ruxton 2005). Studies that at worst control for habitat differences and at best address them experimentally will be required to disentangle the roles of genetics, plasticity and environmental constraints in shaping relationships between traits and driving the evolution of phenotypic integration (Lima & Dill 1990; Murren 2012).

Associations between colour and escape behaviour in Bastimentos but not Dolphin Bay might also suggest that any benefits of conspicuous coloration do not accrue linearly for toxic animals. We built our predictions about how differently coloured morphs would behave based on the assumption that more visually conspicuous morphs pursued a more 'aposematic' strategy, while less conspicuous morphs pursued a more 'cryptic' strategy, an assumption shared by previous work in this genus (Maan & Cummings 2012; Willink et al. 2013). While the assumption that visual conspicuousness can be equated with signal intensity is common, both theoretical (Merilaita & Ruxton 2007) and empirical (Dugas & Rosenthal 2010; Valkonen et al. 2011) examples suggest that the most effective signal need not be the most conspicuous one. Moreover, there seems no compelling reason to assume that the conspicuousness of visual

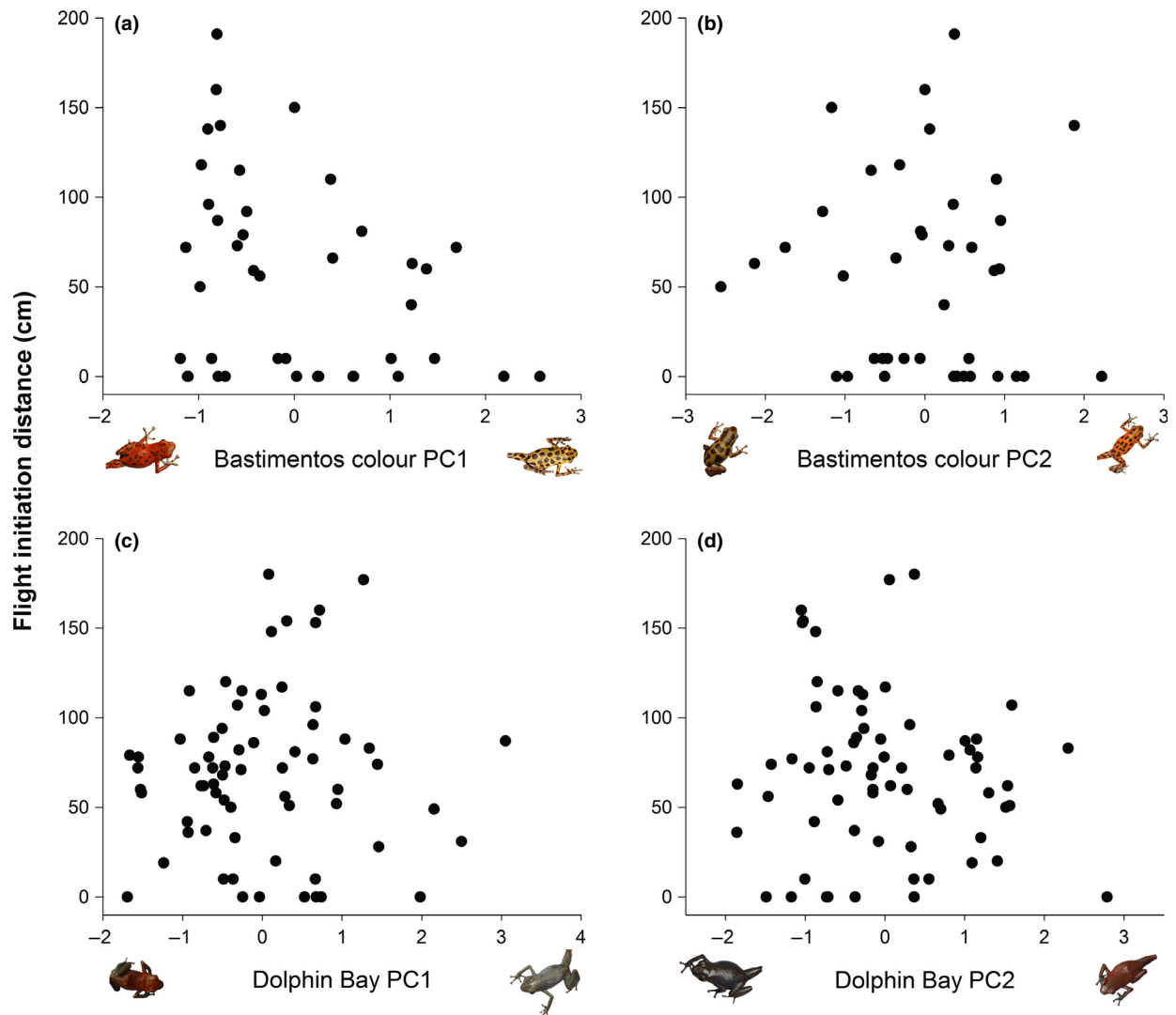


Fig. 4: Relationships between colour and flight initiation distance in *O. pumilio* males from Isla Bastimentos (a, b) and Dolphin Bay (c, d). Principal component (PC) axes were calculated independently for each population. A flight initiation of 0 indicates that the frog did not attempt escape and was captured by hand on the perch.

signals will influence the visual performance of receivers in a linear (vs. threshold) way. Similarly, the extent to which toxicity alone (without aposematic signals) should be associated with boldness remains unclear, as do the levels of toxicity variation that meaningfully influence the behaviour of predators. Although the morphs we studied are visually distinct and differ in conspicuousness and perhaps toxicity (Maan & Cummings 2012), clay model studies suggest that morphs are attacked at equal frequency in both populations (Richards-Zawacki et al. 2013; J. D. Yeager unpubl. data), indicating no relationship between colour and actual protection against predators. Unfortunately, studies like these offer frustratingly little insight into the effects of predators in natural habitats given the

rampant anthropogenic habitat alteration in Bocas del Toro (Pröhl & Ostrowski 2011), and speculation about the function of colours has far outpaced empirical tests (for a rare test supporting an aposematic function of colour, see Saporito et al. 2007b). Improved methodology (Paluh et al. 2014) will aid further assessments of current predation risk, as will identifying (Lenger et al. 2014) and quantifying predators. Aposematism and crypsis are strategies, after all, defined not by colour, but by predator response (Nokelainen et al. 2014).

Deciphering the origin of associations between anti-predator strategies and other behaviours is important for understanding when selection acts on these traits and how complex phenotypes arise (Speed & Ruxton 2005; Murren 2012). Aposematism is an antipredator

strategy that exists on a continuum, and comparing aposematic lineages to more cryptic ones provides a powerful way to test for the hypothesized benefits that signals offer over toxicity alone. Comparisons among polytypic poison frog populations suggest that these benefits may be real, but also highlight their complex origins and the need to consider how traits are integrated not only during evolution, but also during development (Lima & Dill 1990). Predators and prey interact in complex and varied habitats that shape the costs and benefits of both signalling and defensive traits, and future work can develop and test *a priori* hypotheses about the role of the environment in shaping and constraining the expression of these integrated traits. Considering these complicated trade-offs fully will offer further insights into the relationships between behavioural, chemical and morphological evolution.

Acknowledgements

We thank the Smithsonian Tropical Research Institute, in particular S. Castillo, G. Jacome and P. Gondola. Floating Doctors manages the Dolphin Bay site and generously granted access and support. This study was supported by a grant from the National Science Foundation (Award No. 1 146 370) to CLRZ. The Panamanian National Authority for the Environment provided all required permits (No. SE/A-43-14). Heike Pröhl, Walt Koenig and two anonymous reviewers provided helpful comments that improved the quality of this manuscript.

Literature Cited

- Anderson, R. P. & Handley, J. C. O. 2002: Dwarfism in insular sloths: biogeography, selection, and evolutionary rate. *Evolution* **56**, 1045–1058.
- Cooper, W. E. 2003: Effect of risk on aspects of escape behavior by a lizard, *Holbrookia propinqua*, in relation to optimal escape theory. *Ethology* **109**, 617–626.
- Cooper, W. E. 2006: Risk factors affecting escape behaviour by Puerto Rican *Anolis* lizards. *Can. J. Zool.* **84**, 495–504.
- Cooper, W. E., Caldwell, J. P. & Vitt, L. J. 2009a: Risk assessment and withdrawal behavior by two species of aposematic poison frogs, *Dendrobates auratus* and *Oophaga pumilio*, on forest trails. *Ethology* **115**, 311–320.
- Cooper, W. E., Caldwell, J. P. & Vitt, L. J. 2009b: Conspicuousness and vestigial escape behaviour by two dendrobatid frogs, *Dendrobates auratus* and *Oophaga pumilio*. *Behaviour* **146**, 325–351.
- Cooper, W. E. & Avalos, A. 2010: Escape decisions by the syntopic congeners *Sceloporus jarrovi* and *S. virgatus*: comparative effects of perch height and of predator approach speed, persistence, and direction of turning. *J. Herpetol.* **44**, 425–430.
- Dugas, M. B. & Rosenthal, G. G. 2010: Carotenoid-rich mouth colors influence the conspicuousness of nestling birds. *Behav. Ecol. Sociobiol.* **64**, 455–462.
- Graves, B. M. 1999: Diel activity patterns of the sympatric poison dart frogs, *Dendrobates auratus* and *D. pumilio*, in Costa Rica. *J. Herpetol.* **33**, 375–381.
- Hegna, R. H., Saporito, R. A., Gerow, K. G. & Donnelly, M. A. 2011: Contrasting colors of an aposematic poison frog do not affect predation. *Ann. Zool. Fennici* **48**, 29–38.
- Lenger, D. R., Berkey, J. K. & Dugas, M. B. 2014: Predation on the toxic *Oophaga pumilio* (Anura: Dendrobatidae) by *Rhadinaea decorata* (Squamata: Collubridae). *Herpetol. Notes* **7**, 83–84.
- Lessells, C. M. & Boag, P. T. 1987: Unrepeatable repeatabilities: a common mistake. *Auk* **104**, 116–121.
- Lima, S. L. & Dill, L. M. 1990: Behavioral decisions made under risk of predation: a review and prospectus. *Can. J. Zool.* **68**, 619–640.
- Loiseau, C., Fellous, S., Haussy, C., Chastel, O. & Sorci, G. 2008: Condition-dependent effects of corticosterone on a carotenoid-based begging signal in house sparrows. *Horm. Behav.* **53**, 266–273.
- Maan, M. E. & Cummings, M. E. 2008: Female preferences for aposematic signal components in a polymorphic poison frog. *Evolution* **62**, 2334–2345.
- Maan, M. E. & Cummings, M. E. 2012: Poison frog colors are honest signals of toxicity, particularly for bird predators. *Am. Nat.* **179**, E1–E14.
- McVey, M. E., Zahary, R. G., Perry, D. & MacDougal, J. 1981: Territoriality and homing behavior in the poison dart frog (*Dendrobates pumilio*). *Copeia* **1981**, 1–8.
- Merilaita, S. & Ruxton, G. D. 2007: Aposematic signals and the relationship between conspicuousness and distinctiveness. *J. Theo. Biol.* **245**, 268–277.
- Mochida, K. 2009: A parallel geographical mosaic of morphological and behavioural aposematic traits of the newt, *Cynops pyrrhogaster* (Urodela: Salamandridae). *Biol. J. Linn. Soc.* **97**, 613–622.
- Murren, C. J. 2012: The integrated phenotype. *Integr. Comp. Biol.* **52**, 64–76.
- Nokelainen, O., Valkonen, J., Lindstedt, C. & Mappes, J. 2014: Changes in predator community structure shifts the efficacy of two warning signals in Arctiid moths. *J. Anim. Ecol.* **83**, 598–605.
- Paluh, D. J., Hantak, M. M. & Saporito, R. A. 2014: A test of aposematism in the Dendrobatid poison frog *Oophaga pumilio*: the importance of movement in clay model experiments. *J. Herpetol.* **48**, 249–254.
- Perry, G., Wallace, M. C., Perry, D., Curzer, H. & Muhlberger, P. 2011: Toe-clipping of amphibians and reptiles: science, ethics, and the law. *J. Herpetol.* **45**, 547–555.

- Prodanov, D. 2005: RGB Measure Plus (plugin for ImageJ software), <http://rsb.info.nih.gov/ij/plugins/rgb-measure-plus.html>
- Pröhl, H. & Hödl, W. 1999: Parental investment, potential reproductive rates, and mating system in the strawberry dart-poison frog, *Dendrobates pumilio*. *Behav. Ecol. Sociobiol.* **46**, 215–220.
- Pröhl, H. & Ostrowski, T. 2011: Behavioural elements reflect phenotypic colour divergence in a poison frog. *Evol. Ecol.* **25**, 993–1015.
- Rashband, W. S. 1997–2004: ImageJ, U. S. National Institutes of Health, Bethesda, MD, <http://imagej.nih.gov/ij/>
- Richards-Zawacki, C. L., Yeager, J. & Bart, H. P. S. 2013: No evidence for differential survival or predation between sympatric color morphs of an aposematic poison frog. *Evol. Ecol.* **27**, 783–795.
- Rudh, A., Rogell, B. & Höglund, J. 2007: Non-gradual variation in colour morphs of the strawberry poison frog *Dendrobates pumilio*: genetic and geographical isolation suggest a role for selection in maintaining polymorphism. *Molec. Ecol.* **16**, 4284–4294.
- Rudh, A., Rogell, B., Håstad, O. & Qvarnström, A. 2011: Rapid population divergence linked with co-variation between coloration and sexual display in strawberry poison frogs. *Evolution* **65**, 1271–1282.
- Rudh, A., Breed, M. F. & Qvarnström, A. 2013: Does aggression and explorative behaviour decrease with lost warning coloration? *Biol. J. Linn. Soc.* **108**, 116–126.
- Ruxton, G. D., Sherratt, T. N. & Speed, M. P. 2004: *Avoiding attack: the evolutionary ecology of crypsis, warning signals and mimicry*. University Press, Oxford.
- Santos, J. C. & Cannatella, D. C. 2011: Phenotypic integration emerges from aposematism and scale in poison frogs. *Proc. Nat. Acad. Sci. USA* **108**, 6175–6180.
- Saporito, R. A., Donnelly, M. A., Jain, P., Garraffo, H. M., Spande, T. F. & Daly, J. A. 2007b: Spatial and temporal patterns of alkaloid variation in the poison frog *Oophaga pumilio* in Costa Rica and Panama over 30 years. *Toxicon* **50**, 757–778.
- Saporito, R. A., Zuercher, R., Roberts, M., Gerow, K. G. & Donnelly, M. A. 2007a: Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia* **2007**, 1006–1011.
- Speed, M. P. & Ruxton, G. D. 2005: Aposematism: what should our starting point be? *Proc. R. Soc. B* **272**, 431–438.
- Speed, M. P., Brockhurst, M. A. & Ruxton, G. D. 2010: The dual benefits of aposematism: predator avoidance and enhanced resource collection. *Evolution* **64**, 1622–1633.
- Stankowich, T. & Blumstein, D. T. 2005: Fear in animals: a meta-analysis and review of risk assessment. *Proc. R. Soc. B* **272**, 2627–2634.
- Summers, K., Cronin, T. W. & Kennedy, T. 2003: Variation in spectral reflectance among population of *Dendrobates pumilio*, the strawberry poison frog, in the Bocas del Toro Archipelago of Panama. *J. Biogeog.* **30**, 35–53.
- Valkonen, J., Niskanen, M., Bjorklund, M. & Mappes, J. 2011: Disruption or aposematism? Significance of dorsal zigzag pattern of European vipers. *Evol. Ecol.* **25**, 1047–1063.
- Willink, B., Brenes-Mora, E., Bolaños, F. & Pröhl, H. 2013: Not everything is black and white: color and behavioral variation reveal a continuum between cryptic and aposematic strategies in a polymorphic poison frog. *Evolution* **67**, 2783–2794.

Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1. Comparisons of the visual conspicuousness of *O. pumilio* colour morphs used in this study to avian receivers.

Figure S2. Plots of colour principal component 1 by principal component 2 in Bocas del Toro *O. pumilio* populations from Isla Bastimentos (a) and Dolphin Bay (b).

Table S1. Results of a principal components analysis of mean red, green, and blue colour scores from the dorsum of *O. pumilio* from two polymorphic populations in Bocas del Toro, Panama (Bastimentos and Dolphin Bay).

Table S2. Predictors of escape behaviour of male *O. pumilio* from a polymorphic population on Isla Bastimentos, Panama.

Appendix S2. Results of analyses conducted on all observations of male *O. pumilio* in a polymorphic population near Dolphin Bay, on the mainland of Panama.