



The interplay between multiple predators and prey colour divergence

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Evolutionary divergence in the coloration of toxic prey is expected when geographic variation in predator composition and behavior favours shifts in prey conspicuousness. A fundamental prediction of predator-driven colour divergence is that the local coloration should experience lower predation risk than novel prey phenotypes. The dorsal coloration of the granular poison frog varies gradually from populations of conspicuous bright red frogs to populations of dull green and relatively cryptic frogs. We conducted experiments with clay models in four populations to examine the geographic patterns of taxon-specific predation. Birds avoided the local phenotype while lizards consistently selected for decreased conspicuousness and crab predation did not depend on frog coloration. Importantly, birds and lizards favoured low conspicuousness in populations where relatively cryptic green morphs have evolved. This study provides evidence for the interplay among distinct selective pressures, from multiple-predator taxa, acting on the divergence in protective coloration of prey species. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, **113**, 580–589.

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INTRODUCTION

Aposematism evolves by natural selection in prey that benefit from signaling their unprofitability to potential predators (Poulton, 1890; Cott, 1940; Ruxton, Sherratt & Speed, 2004). The design of such warning signals should facilitate predator aversion learning (Guilford & Dawkins, 1991; Speed, 2000) and frequent encounters between signalers and receivers maintain the learned association (Speed, 2000; Ruxton *et al.*, 2004; Mappes, Marples & Endler, 2005). Thus warning signals are expected to be under local stabilizing selection for design uniformity and provide frequency-dependent benefits as common

signals are retained better than rare ones (Endler, 1988; Joron & Mallet, 1998; Lindström *et al.*, 2001). Indeed, empirical work on butterflies and poison frogs has shown that in locally abundant populations of aposematic prey rare and conspicuous morphs (i.e. immigrants or mutants) are strongly selected against (Kapan, 2001; Comeault & Noonan, 2011). For this reason, the maintenance of warning signal polymorphisms and the geographic divergence in warning signals as observed in butterflies, true bugs, moths, and frogs are intriguing topics of current research (e.g. Chamberlain *et al.*, 2009; Fabricant *et al.*, 2013; Willink *et al.*, 2013; Nokelainen *et al.*, 2014). In this study, we investigate the roles of multiple predators and spatially variable predation on the geographic divergence of protective coloration.

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Several studies have suggested that predators maintain the spatial distribution of aposematic coloration in their prey, when they learn to avoid local warning signals but are naïve to the warning signals of phenotypically divergent immigrants (Langham, 2004; Noonan & Comeault, 2009; Chouteau & Angers, 2011). Most studies aimed at understanding the role of predators on signal evolution have focused on the effects of a single predator. Nonetheless, the importance of understanding the combined effects of multiple predators on signal design has been revealed in recent studies. For instance, in aposematic moths spatial heterogeneity in predator composition generates selection mosaics because bird taxa differ in their attack rates on distinct moth morphs (Nokelainen *et al.*, 2014). Similarly, when some predators specialize on defended prey, heterogeneity in predator composition may also turn the balance of selection from favouring to acting against conspicuous warning signals (Valkonen *et al.*, 2012). Taken together, these studies show that predator-specific preferences and predator composition play a role in the maintenance of warning signal polymorphisms and the spatial distribution of aposematic prey. However, the effect of multiple predators on the evolution of geographic divergence in warning signals remains largely unexplored.

The strength of aposematism relies on the extent of the association between warning displays and prey unprofitability. It follows that optimal signal design for a defended prey will vary in relation to predator-specific responses to warning signals and the composition of predator communities. Because predators have diverse visual and cognitive systems a warning signal that enhances prey recognition and aversion learning for one predator might be ineffective for another (Endler, 1988; Speed, 2000). For example, colour patterns that are quickly detected and learned by some bird predators are unmemorable and consistently attacked by others (e.g. Exnerová *et al.*, 2006). In addition, some predators might not be susceptible to prey toxins and select against signal conspicuousness (Endler & Mappes, 2004). Finally, the marginal costs of aposematic displays (e.g. the probability of attracting naïve or tolerant predators and surviving attacks) may depend on the local predator regimes (Speed & Ruxton, 2007; Lindstedt *et al.*, 2011). Thus, conspicuous signaling may not always be beneficial and cryptic or intermediate phenotypes might be better adapted in some contexts. Understanding these dynamics is further complicated by the relative rarity of predation events on aposematic prey, making it difficult to explicitly test the role of predation on phenotypic divergence in prey species.

Aposematic neotropical poison-dart frogs (Dendrobatidae) pose an excellent opportunity to study the role of predators on phenotypic divergence. Bright colours

and toxicity have evolved at least three times within this family (Santos, Coloma & Cannatella, 2003), and some species have subsequently undergone rapid diversification of dorsal colour (Roberts *et al.*, 2006; Wang & Shaffer, 2008; Wang, 2011). In some species dorsal coloration spans a gradient of visual conspicuousness to potential predators (Pröhl & Ostrowski, 2011; Rudh *et al.*, 2011; Willink *et al.*, 2013). For example, the dorsal coloration and visual conspicuousness of the frog *Oophaga granulifera* have diverged along a latitudinal gradient (Wang, 2011; Brusa *et al.*, 2013), with extensive variation across populations, but no distinct local polymorphisms (i.e. discrete morphs) within populations (Willink *et al.*, 2013). In the southern lowlands of Costa Rica and northern Panama frogs have a bright red dorsum. In the northern end of the species distribution frogs are dorsally green. At mid latitudes intermediate frog phenotypes are found spanning the gradient of hues between orange and green, but they differ from both red and green populations when multiple components of colour (i.e. hue, saturation and brightness) are considered (Willink *et al.*, 2013).

Research on the adaptive significance of this colour divergence has been constrained by limited information about the actual predators of poison frogs (for review, see Supporting Information in Santos & Cannatella, 2011). Known predators include snakes (e.g. Michaud & Dixon, 1989; Saporito *et al.*, 2007), tarantulas (Summers, 1999), and a single bird species – i.e. a motmot – (Master, 1999; Alvarado, Alvarez & Saporito, 2013). Despite the scarcity of bird predation records, birds readily attack poison frogs in experimental settings (Poulin *et al.*, 2001; Darst, Cummings & Cannatella 2006). Furthermore, birds possess tetrachromatic colour vision and for bird-specific perceptual models the toxicity and dorsal conspicuousness of *Oophaga pumilio* and *O. granulifera* are correlated across populations (Wang, 2011; Maan & Cummings, 2012). This correlation is positive in *O. pumilio* and negative in *O. granulifera* suggesting that alternative scenarios of predation regimes and prey availability influence the relation between warning signals and defenses, but at least in *O. pumilio* there is no such correlation for the visual systems of other putative predators – i.e. snakes – (Maan & Cummings, 2012). Taken together, these findings have generated the assumptions that birds are the main predators of poison frogs, and that bird predation, reinforced by sexual selection, has driven the evolution of dendrobatid colour variation.

We challenged these assumptions attempting to explicitly identify some natural predators of poison frogs. To do this we monitored clay models of the poison frog *O. granulifera* in the field with camera-traps and conducted predation experiments across the

geographic distribution of colour phenotypes of this species. We examined the geographic and colour dependent attack rates of each predator class (i.e. birds, lizards and crabs). To maintain the geographic distribution of frog coloration and conspicuousness, the combined effects of multiple predators are expected to favour the local phenotype, even if different predators exhibit distinct responses to prey coloration.

MATERIALS AND METHODS

FROG POPULATIONS

Field work was conducted in the western lowlands of Costa Rica, in four populations of *O. granulifera* (Fig. 1). Dorsal colour in this species varies markedly along a latitudinal axis while in all populations frogs display turquoise patches on the limbs and ventral surface which can be more extensive in the southern

red populations. In Palmar Norte and Barú frogs are bright red dorsally and Barú is one of the northernmost dorsally red populations (Brusa *et al.*, 2013). In Portalón, frogs exhibit a variety of intermediate dorsal hues including bronze, orange, yellow-brown, yellow, and yellow-green (Brusa *et al.*, 2013), which are relatively unsaturated and dark (Willink *et al.*, 2013). Finally, San Rafael is located near the northern end of the species distribution (Brusa *et al.*, 2013) and frogs here are dark and unsaturated green (Willink *et al.*, 2013). Of these three colour phenotypes, red, intermediate and green, red frogs are the most conspicuous in terms of both visual contrasts for a model predatory bird and viewer-independent elements of colour space, green frogs are the least conspicuous and intermediate frogs resemble the conspicuous extreme in some traits and the cryptic extreme in others (Willink *et al.*, 2013).

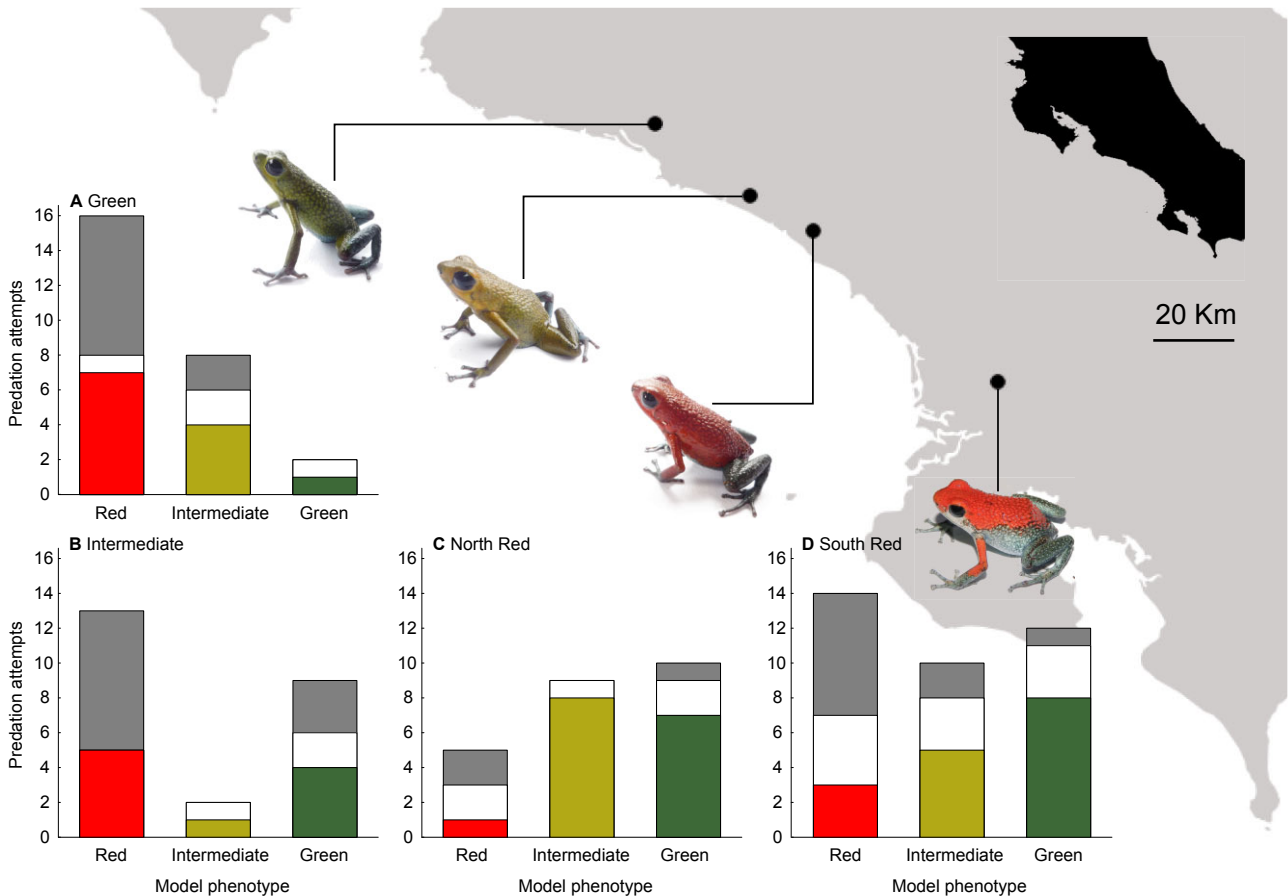


Figure 1. Geographic patterns of colour dependent predation. (Right) Study populations of *O. granulifera* in the western lowlands of Costa Rica. Dorsal frog coloration varies from bright red in the South lowlands of Costa Rica to green in the northern end of the species distribution. In all populations, frogs display variable areas of turquoise coloration on the limbs and venter, usually being more extensive and including the flanks of the body in the southernmost populations. (Left) Number of attacked models of three phenotypes at each population: (A) Green in San Rafael, (B) intermediate in Portalón (C) Northern red in Barú and (D) Southern red in Palmar Norte. Attacks by bird predators are shown in the coloured bars, crab attacks are shown in white and lizard attacks in grey.

PREDATION EXPERIMENTS

Predation experiments were conducted during the rainy seasons (July–November) of 2011 and 2012 with green, representing the northernmost populations, red, representing the southern populations, and yellow-brown, representing an intermediate population, non-toxic clay models of frogs (Supporting Information S1). At each site 900 clay models (300 of each frog phenotype) were placed on trunks, rocks and broad leaves between 0.1–1.0 m above ground since these are likely calling sites of *O. granulifera* males (B. Willink pers. obs.). Frog models were placed in the forested areas on both sides of fast moving streams and only in sections of the streams where frogs naturally occur. Groups of three models (one of each colour) were distributed within 30 m from the water (wherever the landscape allowed), and were separated by at least 4 m. Each model was 2 m apart from the other two models in a group. At each locality models were placed on three days, 300 models per day. After 72 h, models were collected and inspected for predation marks. Attacked models were photographed and marks on the clay were used to identify the predator taxon as shown in Figure 2. Birds were characterized by leaving parallel deep incisions on the flanks of the model (Fig. 2F, K), stabs on the dorsum (Fig. 2G, L), or V- or U-shaped marks on the dorsal and ventral surface of models (Fig. 2H, M). Lizard marks were also U- or V- shaped but teeth punctures were readily visible (Fig. 2I, N). Crab predation attempts were identified in the field as they usually dragged the models to the entrance of underground burrows, or under rocks or logs. These models exhibited a variety of marks, from one to multiple deep cuts (Fig. 2J) to a completely torn-into-pieces model (Fig. 2O). Attacks by unknown assailants included models with missing limbs, limbs with missing bodies, and disfigured models with no recognizable beak, teeth or pincers marks.

CAMERA-TRAP TRIALS

To aid the interpretation of predation marks we conducted additional trials in the North-Red population at the Firestone Center for Restoration Ecology and Hacienda Barú. For these trials three models were placed close together – usually within 0.30 m – and a Bushnell Trophy Cam camera-trap was attached to a tree in front of the clay models at a distance of no more than 1.5 m. The cameras were embedded in bark-colour metallic armors and used infrared flash to reduce the probability of alarming predators. Camera-traps were set to high sensitivity for motion triggering to record as many potential predators as possible. However, during preliminary trials we found that motion sensors were rarely activated by reptiles

and large arthropods. Thus we set the camera-traps to additionally record a 10 s video every minute from 5:00 to 17:30; the period comprising the light hours in the forest understory. Cameras scanned the models for up to 72 h, when models were collected and inspected and videos were downloaded. We conducted 14 of these trials, each with 11–26 camera-traps. Aiming to sample a wide variety of potential predators, we conducted trials in four sites, three in the forests surrounding streams and one in an abandoned banana plantation where frogs are abundant. Three to four trials were conducted before the cameras were moved to a different site. Overall, we monitored 260 groups of models, recording a total of 4665 h.

STATISTICAL ANALYSIS

Predation data by birds, lizards and crabs were analyzed using Generalized Linear Mixed Models (GLMMs). A binomial error distribution was assumed because each frog was scored as attacked or intact. We used model colour and origin (i.e. local or novel) as explanatory variables to test for two possible scenarios: (1) that a certain colour morph experiences a selective advantage or disadvantage across populations, and (2) that local phenotypes are better protected. A significant interaction would mean that the relative advantage of the local phenotype depends on its colour, for instance if novelty is less disadvantageous for the more cryptic phenotypes, or alternatively if conspicuous warning signals enhance the protection of the local morph. We included the frog population as a random variable to account for our spatial sampling structure. We report association tests between the probability of being attacked and each factor. Missing models were excluded from the analysis because it is likely that most of these models were lost during rainstorms. Rainstorms occurred at least once during every experiment, and the rain and wind could drag models downhill up to several meters.

RESULTS AND DISCUSSION

We conducted predation experiments in four populations of *O. granulifera*, spanning nearly its entire coloration range, expecting that local phenotypes would experience a selective advantage. While attacks on clay models were rare at all sites three predator classes, namely birds, lizards and crabs were involved in the attacks (Figs 1, 2). To confirm the interpretation of predation marks in these experiments we compared them to marks from camera-trap identified predators. During the camera-trap trials, 29 frog models were attacked, and 11 of these predators were recorded. The other 18 assailants failed to trigger the camera sensors and damaged the models



Figure 2. Frog predators and attacked models. (A–E) Five of the predators that attacked clay models of *O. granulifera*: (A) *Momotus momota*, (B) *Crax rubra*, (C) *Tinamus major*, (D) *Basiliscus basiliscus*, (E) Pseudothelphusid crab from Costa Rica; the species attacking the models are unknown. Photographs courtesy of C. Jiménez (A), D. Sigüenza, (B), M. Calderón (C), V. Acosta (D) and E. Boza (E). (F–J) Examples of marks on clay respectively inflicted by the recorded predators above: (F) incisions on the flanks, (G) stabs on the dorsum, (H) U-shaped beak mark, (I) U-shaped mark with teeth punctures, (J) pincers grasp on the head of a model. (K–O) Examples of predation marks assigned to a predator taxa: (K–M) birds, (N) lizard, (O) crab. This model was found in the entrance of an underground burrow.

between scanning periods or at night (See Materials and Methods for details). Recorded predators included birds, lizards, and a crab (Fig. 2A–E, Supporting Information S2), but some diurnal mammals, white-nosed coatis, a white-faced capuchin and a collard peccary were attracted to and inspected the models (Supporting Information S2, Video S1).

In total, 110 models were attacked by recognizable predators, which represents roughly 3% of the 3600 available models (frog populations varying in attack rates from 2.7% to 4.0%, Fig. 1). Birds preyed upon 1.6% of the recovered models, lizards on 1.0%, and crabs on 0.7%. Previous studies supporting predator-maintained aposematism in poison frogs report higher (3–12%) overall rates of predation attempts by birds (Noonan & Comeault, 2009; Chouteau & Angers, 2011; Hegna *et al.*, 2011). However, our results are consistent with low bird predation rates (1.5%) observed in an island population of *Oophaga pumilio* where the local phenotype actually suffered higher bird predation than a non-local morph (Hegna, Saporito & Donnelly, 2013). Such low predation rates, especially if in conflict with an adaptive hypothesis, have been the reason to argue that genetic drift and sexual selection drive of the evolution of geographic colour divergence in some poison frog species (Chouteau & Angers, 2012; Hegna *et al.*, 2013). Nonetheless divergent selection by predators may be involved in the latitudinal phenotypic gradient in *O. granulifera*, given that frog populations also differ gradually in toxicity, visual conspicuousness, and behavior (Wang, 2011; Willink *et al.*, 2013). Current evidence indicates that the intermediate and green morphs are derived from the ancestral red phenotype, and these populations probably resulted of the range expansion of the species to the North (Wang, 2011). Thus, from South to North *O. granulifera* evolved increased toxicity and decreased conspicuousness in terms of both behavior (e.g. calling activity, movement and escape behavior) and visual contrasts in their environments, suggesting evolutionary changes in predator avoidance strategies towards higher reliance on crypsis (Willink *et al.*, 2013).

Our results support the idea of divergent selection by predators in spite of their distinct responses to frog colour morphs that appear to reflect their systems for detecting, discriminating, and learning to recognize

Table 1. Results of GLMMs fitted on predation data. We report association tests between the probability of being attacked by birds, lizards and crabs and the colour (red, intermediate, or green) and origin (local or novel) of frog models

Explanatory term	χ^2	DF	P-value
Bird attacks			
Colour	0.053	2	0.974
Origin	9.272	1	0.002
Colour*Origin	0.472	2	0.790
Lizard attacks			
Colour	15.853	2	<0.001
Origin	2.147	1	0.143
Colour*Origin	0.001	2	0.999
Crab attacks			
Colour	0.026	2	0.872
Origin	0.028	1	0.986
Colour*Origin	3.609	2	0.164
All predators			
Colour	8.136	2	0.004
Origin	6.859	1	0.032
Colour*Origin	3.328	2	0.189

Significant effects ($\alpha = 0.05$) are in bold.

warning signals. For instance, bird attacks are indicative of predators endowed with colour vision and the ability to learn to avoid noxious prey. Bird predation was lower on the local colour morph regardless of its phenotype (Table 1, Fig. 1). Birds are known to use conspicuousness to discern unpalatable prey through aversion learning (Gittleman & Harvey, 1980; Guilford, 1986). However, it is also possible that birds have learned to avoid the green and intermediate morphs where local given that birds can learn and discriminate specific hues, colour patterns or shapes even if relatively inconspicuous (e.g. Osorio, Jones & Vorobyev, 1999; Valkonen, Nokelainen & Mappes, 2011; Valkonen *et al.*, 2012). In addition, bird aversion learning of the less conspicuous forms can be favoured by their higher toxicity. Darst *et al.* (2006) demonstrated that signal strength and defense level can be combined in different ways to achieve similar protection from bird predators. Specifically, they found that in a pair of poison-frog species highly conspicu-

ous warning signals in combination with moderate toxicity, and moderately conspicuous signals with high toxicity yielded similarly high protection from avian predators.

An alternative explanation for the elevated protection of less conspicuous morphs where they are local is that in these populations crypsis may play a more important role in predator deterrence than learned aversion. Aversion learning in predators is likely influenced by encounter rates with warning signals. Encounters with defended prey that are not followed by an attack can potentially jog (i.e. reactivate) the memories of experienced predators and preclude, reverse or delay forgetting (Speed, 2000). Theory predicts that encounter rates with predators should exceed a certain threshold for predators to retain the association between the warning signal and the noxious consequences of a predation attempt (Endler & Rojas, 2009). We believe that encounter rates may be lower in the northern populations for three reasons. First, warning signals are less conspicuous. Green frogs are more visually similar to their backgrounds and also call less, move less and hide more (Willink *et al.*, 2013), so they should be less frequently detected by a nearby potential predator. Second, frog density probably decreases from South to North as we observed fewer frogs of the less conspicuous morph in similarly long transects of predation experiments (B. Willink pers. obs.). Third, in a preliminary study of frog distribution, we found that in a green population male frog territories were always clumped in groups of 2–3 individuals (males within a clumped separated by 1–6 m, clumps separated by 12–30 m, $n = 11$ males), whereas no identifiable clumps (i.e. groups of frogs separated by more than 12 m) were found in a red population ($n = 31$ males) and only 8% of males formed such clumps in an intermediate population ($n = 25$ males) (B. Willink unpublished data). A tightly aggregated dispersion of prey leads to spatial variation in the response of predators to warning signals (Endler & Rojas, 2009). Put in other words, with higher aggregation relatively fewer predators are expected to learn and remember the warning signal association. Thus, the combination of low conspicuousness, low density and tightly aggregated dispersion indicates that at least in the green population protection of the local morph may be a result of relative crypsis rather than discrimination by educated predators.

On the other hand, lizard predation was governed solely by prey phenotype, while there was no effect of model origin or interactions between both factors (Table 1). Lizards attacked red models with the highest frequency in all populations except the North Red which had low overall lizard predation (Fig. 1). Diurnal lizards, like birds, tend to have good

tetrachromatic colour vision (e.g. Barbour *et al.*, 2002; Loew *et al.*, 2002). Assuming that lizards perceive visual contrasts in a fashion similar to birds, red frog models would be the most likely to be encountered as they have the highest contrasts against natural backgrounds for bird vision (Willink *et al.*, 2013). This implies that lizards cannot associate warning signals with distastefulness, or that they have low susceptibility to poison frog toxicity. Alternatively, lizards may be able to associate intermediate and green colorations with distastefulness because the toxicity of such phenotypes exceeds a certain threshold, but fail to do so in red populations, which are less toxic. While some studies show that lizards can learn to avoid distasteful prey (Boyden, 1976; Sword *et al.*, 2000; Tseng *et al.*, 2014), and that learning is enhanced by conspicuous colorations (Boyden, 1976), lizards likely use both, visual signals (present in clay models) and olfactory cues (absent in clay models) to assess prey palatability (Sword *et al.*, 2000). Indeed in a recent study, lizards increased their attack rates on aposematic beetles when prey colour patterns were concealed, but they attacked less manipulated prey than inexperienced lizards suggesting that additional cues mediate avoidance learning and recognition of aposematic prey in this system (Tseng *et al.*, 2014). Our study with visual signals points to selection against the red and highly conspicuous phenotype in the northern and intermediate populations through the combined effects of bird and lizard predation. In future studies addressing lizard predation the whole suite of cues for toxicity available in natural settings should be examined.

Crabs exhibited a third form of predation that seemed not to depend on either the colour or the origin of frog models (Table 1, Fig. 1). However, this result must be taken cautiously due to the very low rate of crab attacks and hence reduced statistical power in our analysis. Crabs have recently been acknowledged as potential predators exerting selection on the coloration of poison frogs because at least some crabs are able to see colours (Horch, Salmon & Forward, 2002) and because for one crab visual system (the fiddler crabs in the family Ocypodidae) the dorsal conspicuousness of *O. pumilio* is correlated with toxicity across colour morphs (Maan & Cummings, 2012). The ecological relevance of this association is yet to be confirmed since it has not been tested for the visual system of crab taxa which can actually encounter and prey on poison frogs. For instance the neotropical freshwater crabs (Pseudothelphusidae), which attacked models in this study, can be opportunistic predators on terrestrial prey (in contrast to other crabs that are detritivorous scavengers) (Maitland, 2003; Yeo *et al.*, 2008) and forage mostly at night when warning colorations may be less detectable.

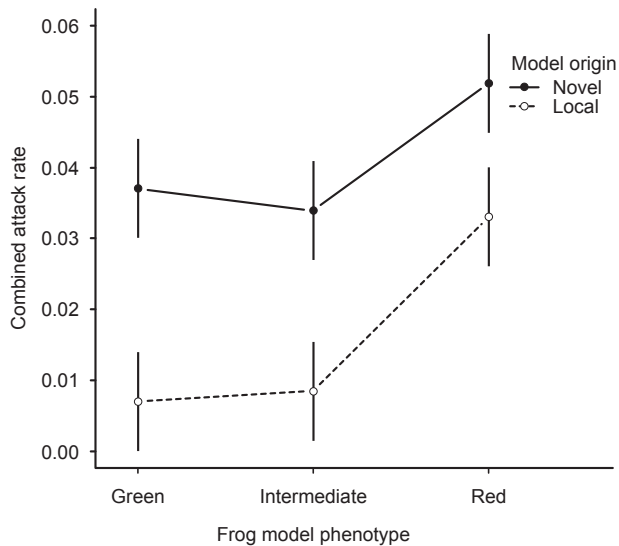


Figure 3. Mean attack rates (attacks in 72 h/model) by avian, lizard and crab predators combined on clay models of three colour morphs of *O. granulifera*. Bars represent standard errors.

Thus while freshwater crabs may attack poison frogs, we still are far from understanding how they perceive and evaluate warning signals.

Finally, when the responses of all three predator types are treated as one single selective pressure there is an overall advantage to the local morph (Table 1), and higher total predation on the red phenotype, compared to the green and intermediate morphs (Table 1, Fig. 3). This latter result arises from the difference in local phenotype protection between the two red populations studied. Multiple-predator selection appears to favour the local red morph only in the Northern Red population (Fig. 1c, d). Population admixture analyses in *O. granulifera* suggest higher gene flow across the Northern Red, intermediate and green populations than between both of our red populations (Brusa *et al.*, 2013). Thus, a stronger role of predator-driven divergent selection within the area of high gene flow would be expected for the maintenance of phenotypic divergence (reviewed in Nosil, 2012). Of course, this interpretation assumes that predator attack rates on clay models reflect the strength of selection on frog coloration. However, predator preferences alone may be a weak predictor of such selection if predators also differ in their abilities to capture and handle prey. Future work assessing the effects of multiple predators on warning signal evolution should address capture success in addition to predator preferences, especially if divergence in warning signals is associated with divergence in predator avoidance behavior as in *O. granulifera* (Willink *et al.*, 2013).

Our results uncover different patterns of colour dependent attack rates on models of *O. granulifera* by different predator taxa. We show that bird predation favours the geographic distribution of dorsal coloration and lizard predation strengthens selection against the red phenotype in the North. These results illustrate how the impact of predator communities on the evolution of prey warning signals may differ from the effects of a single predator type. Had the effects of lizard predation been overlooked in this system, selection against the red morph in the northern populations would have been underestimated. Predator communities have the potential of influencing the evolution of prey warning signals in multiple ways. Different perception abilities of predators may favour different modalities of warning signals (Ratcliffe & Nydam, 2008) or different levels of signal conspicuousness (Mochida, 2011). Additionally, variable tolerance to chemical defenses among predators affect the level of optimal signal conspicuousness, because prey should always avoid encounters with tolerant predators (Endler & Mappes, 2004). Understanding these effects becomes even more challenging when the community ecology of predators is considered. Variation in the relative abundance (Valkonen *et al.*, 2012; Nokelainen *et al.*, 2014) and spatial distribution (Endler & Rojas, 2009) of predators further influences how natural selection acts on prey warning signals. Our study highlights the importance of expanding our knowledge on the natural predators of current and prospective model systems by providing direct evidence for the different effects of multiple predators on the divergence in protective coloration of prey species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

S1. Construction of frog clay models

S2. Potential predators of the granular poison frog *Oophaga granulifera*

Video S1. White-nosed coati (*Nasua narica*) approaching and smelling clay models of the granular poison (*Oophaga granulifera*). The models were not attacked.

Video S2. Lizard (*Basiliscus basiliscus*) attack on a clay model of an intermediate colour morph of the granular poison (*Oophaga granulifera*). The lizard pecked on the model after being nearby for several minutes.

Video S3. Motmot (*Momotus momota*) attack on a clay model of the red colour morph of the granular poison (*Oophaga granulifera*). The bird flew with the model in its beak to another branch and dropped it after a few seconds.

Video S4. Great Tinamu (*Tinamus major*) recorded seconds after attacking a red clay model of the granular poison (*Oophaga granulifera*). By the time the camera was triggered the red model was already on the floor.

Video S5. Swainson's thrush (*Catharus ustulatus*) examining a clay model of an intermediate colour morph of the granular poison (*Oophaga granulifera*) before pecking on it a single time.