biology
letters

The role of predator selection on polymorphic aposematic poison frogs

Brice P Noonan and Aaron A Comeault

Biol. Lett. 2009 **5**, 51-54
doi: 10.1098/rsbl.2008.0586

biology letters

Biol. Lett. (2009) **5**, 51–54
doi:10.1098/rsbl.2008.0586

Evolutionary biology

The role of predator selection on polymorphic aposematic poison frogs

Brice P. Noonan* **and Aaron A. Comeault**

Department of Biology, University of Mississippi, University, MS 38677, USA
**Author for correspondence* (*bnoonan@olemiss.edu*).

Demonstrations of interactions between diverse selective forces on bright coloration in defended species are rare. Recent work has suggested that not only do the bright colours of Neotropical poison frogs serve to deter predators, but they also play a role in sexual selection, with females preferring males similar to themselves. These studies report an interaction between the selective forces of mate choice and predation. However, evidence demonstrating phenotypic discrimination by potential predators on these polymorphic species is lacking. The possibility remains that visual (avian) predators possess an inherent avoidance of brightly coloured diurnal anurans and purifying selection against novel phenotypes within populations is due solely to non-random mating. Here, we examine the influence of predation on phenotypic variation in a polymorphic species of poison frog, *Dendrobates tinctorius*. Using clay models, we demonstrate a purifying role for predator selection, as brightly coloured novel forms are more likely to suffer an attack than both local aposematic and cryptic forms. Additionally, local aposematic forms are attacked, though infrequently, indicating ongoing testing/learning and a lack of innate avoidance. These results demonstrate predator-driven phenotypic purification within populations and suggest colour patterns of poison frogs may truly represent a 'magic trait'.

Keywords: aposematic; selection; *Dendrobates*; magic trait

1. INTRODUCTION

The consideration of the respective roles of sexual and predator selection in driving the evolution of bright coloration dates to a series of letters between Charles Darwin and Alfred R. Wallace in late February of 1867. Since that time, the study of sexual selection and aposematic coloration has flourished, clearly illustrating the impact of mate choice and predator–prey interactions in shaping the phenotype of many organisms. Numerous studies have documented the role that sexual selection exerts on aspects of the phenotype (Summers *et al.* 1999; Reynolds & Fitzpatrick 2007; Maan & Cummings 2008). So too has the study of protected prey revealed the ability of predators to recognize and avoid unprofitable prey items based on their coloration, pattern or some combination of the two (Brodie 1993; Langham 2004; Kuchta 2005; Ham *et al.* 2006; Saporito *et al.* 2007).

Though aposematic coloration is relatively uncommon among vertebrates, the diverse Neotropical poison frogs (Dendrobatidae) have repeatedly evolved warning coloration and the ability to cutaneously sequester unpalatable alkaloids from their prey (Santos *et al.* 2003; Vences *et al.* 2003). One of the most puzzling aspects of conspicuous coloration/pattern is geographically structured intraspecific variation (Summers *et al.* 1997). Such variation in chemically protected butterflies, for example, has fuelled decades of research into aspects of speciation, mimicry and chemical defence. Among the Dendrobatidae, there are a number of species that, throughout their distributions, exhibit phenotypic variation rivalling that of any polymorphic aposematic species. Among these species, populations of *Oophaga pumilio* in Costa Rica and Panama have been the subject of numerous studies investigating the role of coloration in mate choice and protection from predation (Summers *et al.* 1997, 1999; Siddiqui *et al.* 2004; Reynolds & Fitzpatrick 2007; Saporito *et al.* 2007; Maan & Cummings 2008).

Reynolds & Fitzpatrick (2007) examined the mating preference of individual poison frogs with varying phenotypes, citing their findings (preference for similar individuals) as an example of an interaction between the forces of predator selection and mate choice on a single trait. Maan & Cummings (2008) report results in which females occasionally choose novel-patterned mates, suggesting a role for both mate and predator bias in shaping phenotypic variation at the population level. Combined, these findings indicate that the colour pattern of poison frogs may represent one of the few known examples of a 'magic trait', one subject to both disruptive selection and assortative mating (Gavrilets 2004). Although predator avoidance of warningly coloured frogs has been demonstrated in the field (Saporito *et al.* 2007), it is unclear whether predators discriminate among differently patterned individuals of brightly coloured species. It is important to note that here disruptive selection is the 'broader notion' of the phenomenon described by Gavrilets (2004, p. 234) in which different parts of the population may experience selection in different directions.

Alternatively, novel phenotypes may arise and persist due to apostatic or innate avoidance by predators. Götmark (1996) found wild avian predators avoided novel, conspicuous prey (apostatic selection; but see Lindström *et al.* 2001*a*,*b*; Langham 2004). It is also possible that the combination of bright coloration (of any sort) combined with diurnal activity is a sufficient signal to deter potential predators (Siddiqui *et al.* 2004). Thus, it may be that sexual selection alone is driving geographically structured, intraspecific variation observed in these dendrobatid frogs.

We tested the influence of predator selection on phenotypic variation within the range of one such variable species of dendrobatid, *Dendrobates tinctorius*. This species is endemic to the Guiana Shield and exhibits significant geographical variation in aspects of both colour and pattern (figure 1) despite significant gene flow (Noonan & Gaucher 2006). Though

Received 7 October 2008
Accepted 24 October 2008

Figure 1. (*a*) Local and (*b*) novel phenotypes of *D. tinctorius* upon which (*c*,*e*) models, respectively, were modelled; (*d*) cryptic model.

mate choice experiments have not been conducted in this species, females, which do not consistently differ from males in any phenotypic characteristic, actively approach and court males (Lötters *et al.* 2007; B.P. Noonan 2008, personal observation) and some measure of assortative mating similar to that observed in *Oophaga* is probable. In order to examine ecological forces on polymorphic dendrobatids, we used clay models placed throughout primary forest inhabited by a single phenotypic form of this species, examining patterns of predation on models resembling the local form, a novel form (resembling populations approx. 150 km away) and a cryptic (brown) form.

2. MATERIAL AND METHODS

Models were constructed by pouring melted Van Aken modelling clay into moulds of a toy model of *D. tinctorius*. This clay is well suited to this type of study as it does not harden and retains markings left by predators. We modelled forms phenotypically similar to populations found in the test site of Saul, French Guiana (yellow dorsum, black legs, figure 1*a*,*c*) and a coastal population approximately 150 km northeast of Saul (blue legs, black dorsum, yellow stripes on dorsum, figure 1*b*,*e*) as well as a cryptic, brown form (figure 1*d*). Pre-coloured clay was used to produce all aspects of colour pattern. As both dendrobatid frogs and clay have been shown to lack significant UV reflectance (Saporito *et al.* 2007), clay colours were matched by eye. Models were 45 mm in length (snout vent), similar to the size of individuals from the sampled population.

In order to test selection on cryptic, local and novel phenotypes, we placed models directly on the leaf litter along 6.3 km of transects near Saul, French Guiana (Mt Boeuf Mort), in two segments (4.2 and 2.1 km), between 5 and 14 July. Transects were placed in 1.05 km increments, sequentially (temporally and spatially) around the mountain with the two segments separated by no less than 2.5 km at all times. We did not attempt to compensate for the relative crypsis of the three models, as previous studies of aposematic snakes (Brodie 1993) and frogs (Saporito *et al.* 2007) have demonstrated that both cryptic and aposematic models are attacked more frequently when placed directly on the leaf litter rather than a high contrast background. Transects were divided into two 525 m long segments with models randomly (www.random.org) placed every 5 m (105 total models, 35 of each form). A total of 1260 models were placed along 6.3 km of transects in the forest for 72 hours (a total of 3780 model days). After 72 hours, models were collected, inspected and individuals exhibiting evidence of attack were photographed. To avoid disturbance, we did not visit transects between deposition and collection. Attacks were categorized as bird, rodent or unknown. The latter category did not include those markings attributable to insects (e.g. ants or beetles). Models not recovered (10) were scored as missing and not included in analysis. Following Brodie (1993), consecutive attacks were scored as a single event. The *G*-test of independence was used to determine whether coloration was a predictor of predation risk.

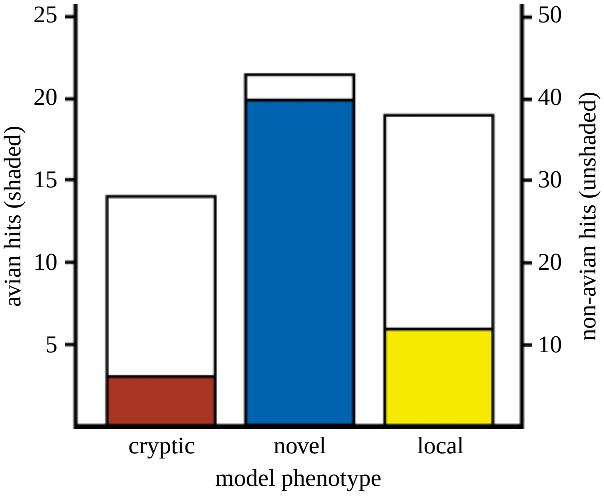

Figure 2. Frequency of cryptic (brown), novel (blue) and local (yellow) models attacked by visual (avian) and non-visual predators.

3. RESULTS

Of our 1260 models, 139 (11%) were attacked. Attacks attributable to mammals (primarily rodents) and unknown assailants (60 and 30, respectively) made up the vast majority of attacks. Twenty-nine attacks (21% of total) were attributable to visual (avian) predators. Phenotype was not a significant predictor of non-avian predation ($G=2.42$, $p=0.299$; figure 2). However, avian predation was strongly associated with phenotype ($G = 15.84$, $p < 0.001$) with the novel aposematic phenotype being attacked significantly more frequently than both the cryptic ($G = 13.54$, $p < 0.001$) and local aposematic forms ($G = 7.758$, $p = 0.005$; figure 2).

4. DISCUSSION

Our results clearly demonstrate a selective advantage of local aposematic phenotypes over novel forms in deterring predation on poison frogs. Novel brightly coloured models were more than three times as likely to be attacked by an avian predator as local aposematic models. For *D. tinctorius*, these findings are particularly interesting as populations are discretely distributed on mountainous/hilly uplands and frequently quite isolated from one another by uninhabited lowland areas (Noonan & Gaucher 2006). This would suggest that the difficulties of inter-patch dispersal are compounded by selective disadvantages encountered by migrants in the form of high predation risk and possible low reproductive success.

Though it may be argued that the higher rate of attack on blue models may be due to the relative conspicuousness of the two brightly coloured models, preliminary data from a small number of reciprocal transects in a population (Nouragues) phenotypically similar to the blue model indicate that this is not the case. While we have comparatively few transects from this locality (405 models), avian attacks were again significantly more frequent on the novel (yellow) model relative to the local model (blue; $G = 5.0$, $p < 0.025$). These data indicate that predator sampling bias is the result of avoidance of local aposematic phenotypes in each population rather than selection for the most conspicuous form.

Though we did observe more avian attacks on the local aposematic form (yellow, five) than the cryptic form (brown, two), these results were not significant ($G = 0.97$, $p = 0.33$). That yellow models were attacked at all suggests a component of ongoing learning and/or continued testing of local forms and an absence of innate avoidance of brightly coloured diurnal anurans. This continued sampling of local aposematic forms and the high sampling rate of novel forms confirm previous studies demonstrating the purifying effects of predator selection on phenotype (Gamberale-Stille & Guilford 2004) and reject a handicap function (Gamberale-Stille & Guilford 2003) of the coloration of dendrobatid frogs.

Our results provide complementary support to previous conclusions of interaction (pleiotropy *sensu* Smith (1966)) between sexual and predator selection (Summers *et al.* 1997; Reynolds & Fitzpatrick 2007; Maan & Cummings 2008) on phenotypic variation in Neotropical poison frogs and counter suggestions that phenotypic variation in poison frogs may be irrelevant in deterring predators (Siddiqui *et al.* 2004). Observed patterns of predation suggest novel phenotypes within populations experience a significant selective disadvantage (Lindström 2001*b*; contra Götmark 1996). However, the patchy distribution of *D. tinctorius* appears to be particularly well suited to the establishment of novel forms with slight biases in female preferences of founding propagules being exacerbated and quickly fixed by predators.

We would like to thank Molly Cummings, Philippe Gaucher, Ralph Saporito and particularly Justin Yeager for their thoughtful discussions of these experiments. David Reed, Jason Hokesema and two anonymous reviewers provided helpful comments on earlier versions of the manuscript. Fieldwork in French Guiana was supported in part by a Nouragues grant from CNRS.

Brodie III, E. D. 1993 Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa Rica. *Evolution* **47**, 227–235. (doi:10.2307/2410131)

Gamberale-Stille, G. & Guilford, T. 2003 Contrast versus color in aposematic signals. *Anim. Behav.* **65**, 1021–1026. (doi:10.1006/anbe.2003.2098)

Gamberale-Stille, G. & Guilford, T. 2004 Automimicry destabilizes aposematism: predator sample-and-reject behaviour may provide a solution. *Proc. R. Soc. B* **271**, 2621–2625. (doi:10.1098/rspb.2004.2893)

Gavrilets, S. 2004 *Fitness landscapes and the origin of species.* Princeton, NJ: Princeton University Press.

Götmark, F. 1996 Simulating a colour mutation: conspicuous red wings in the European blackbird reduce the risk of attacks by sparrowhawks. *Func. Ecol.* **10**, 355–359. (doi:10.2307/2390283)

Ham, A. D., Ihalainen, E., Lindström, L. & Mappes, J. 2006 Does colour matter? The importance of colour in avoidance learning, memorability and generalization. *Behav. Ecol. Sociobiol.* **60**, 482–491. (doi:10.1007/s00265-006-0190-4)

Kuchta, S. R. 2005 Experimental support for aposematic coloration in the salamander *Ensatina eschscholtzii xanthoptica*: implications for mimicry of Pacific Newts. *Copeia* **2002**, 265–271. (doi:10.1643/CH-04-173R)

Langham, G. M. 2004 Specialized avian predators repeatedly attack novel color morphs of *Heliconius* butterflies. *Evolution* **58**, 2783–2787. (doi:10.1554/04-207)

Lindström, L., Alatalo, R. V., Lyytinen, A. & Mappes, J. 2001*a* Predator experience on cryptic prey affects the survival of conspicuous aposematic prey. *Proc. R. Soc. B* **268**, 357–361. (doi:10.1098/rspb.2000.1377)

Lindström, L., Alatalo, R. V., Lyytinen, A. & Mappes, J. 2001*b* Strong antiapostatic selection against novel rare aposematic prey. *Proc. Natl. Acad. Sci. USA* **98**, 9181–9184. (doi:10.1073/pnas.161071598)

Lötters, S., Jungfer, K.-H., Henkel, F. W. & Schmidt, W. 2007 *Poison frogs. Biology, species & captive husbandry.* Frankfurt am Main, Germany: Edition Chimaira.

Maan, M. E. & Cummings, M. E. 2008 Female preferences for aposematic signal components in a polymorphic poison frog. *Evolution* **62**, 2334–2345. (doi:10.1111/j.1558-5646.2008.00454.x)

Noonan, B. P. & Gaucher, P. 2006 Refugial isolation and secondary contact in the dyeing poison frog *Dendrobates tinctorius*. *Mol. Ecol.* **15**, 4425–4435. (doi: 10.1111/j.1365-294X.2006.03074.x)

Reynolds, R. G. & Fitzpatrick, B. M. 2007 Assortative mating in poison-dart frogs based on an ecologically important trait. *Evolution* **61**, 2253–2259. (doi:10.1111/j.1558-5646.2007.00174x)

Santos, J. C., Coloma, L. A. & Cannatella, D. C. 2003 Multiple, recurring origins of aposematism and diet specialization in poison frogs. *Proc. Natl. Acad. Sci. USA* **100**, 12 792–12 797. (doi:10.1073/pnas.2133521100)

Saporito, R. A., Zuercher, R., Roberts, M., Gerow, K. G. & Donnelly, M. A. 2007 Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia* **2007**, 1006–1011. (doi:10.1643/0045-8511(2007)7[1006:EEFAIT]2.0.CO;2)

Siddiqi, A., Cronin, T. W., Loew, E. R., Vorobyev, M. & Summers, K. 2004 Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. *J. Exp. Biol.* **207**, 2471–2485. (doi:10.1242/jeb.01047)

Smith, J. M. 1966 Sympatric speciation. *Am. Nat.* **100**, 637–650. (doi:10.1086/282457)

Summers, K., Bermingham, E., Weigt, L., McCafferty, S. & Dahlstrom, L. 1997 Phenotypic and genetic divergence in three species of dart-poison frogs with contrasting parental behavior. *J. Hered.* **88**, 8–13.

Summers, K., Symula, R., Clough, M. & Cronin, T. 1999 Visual mate choice in poison frogs. *Proc. R. Soc. B* **266**, 2141–2145. (doi:10.1098/rspb.1999.0900)

Vences, M., Kosuch, J., Boistel, R., Haddad, C. F. B., La Marca, E., Lötters, S. & Veith, M. 2003 Convergent evolution of aposematic coloration in neotropical poison frogs: a molecular phylogenetic perspective. *Org. Divers. Evol.* **3**, 215–226. (doi:1439-6092/03/03/03-215)