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Preferences for and use of light microhabitats differ among and within populations of a polytypic poison frog

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Anti-predator strategies can influence trade-offs governing other activities important to fitness. Crypsis, for example, might make conspicuous sexual display especially costly, whereas aposematism might reduce or remove such costs. We tested for correlates of anti-predator strategy in *Oophaga pumilio*, a polytypic poison frog with morphs spanning the crypsis–aposematism continuum. In the wild, males of visually conspicuous morphs display from conspicuous perches and behave as if they perceive predation risk to be low. We thus predicted that, given a choice of ambient light microhabitats, these males would use high ambient light conditions the most and be most likely to perch in high-light conditions. We found no evidence that differently colored male *O. pumilio* preferentially used bright microhabitats or that ambient light influenced perching in a morph-specific manner. Independent of light conditions, males from the most conspicuous population perched the least, but the most conspicuous individuals from a polymorphic population perched the most. These patterns suggest that preferences do not necessarily underlie among-morph differences observed in the wild. This could be explained, and remain consistent with theory, if risk aversion is shaped, in part, by experience.

ADDITIONAL KEYWORDS: aposematism – colour polytypism – crypsis – Dendrobatidae – integrated phenotype.

INTRODUCTION

The intensity and direction of selection on any one trait often depend in complex and/or context-dependent ways on the expression of other traits in the same individual, driving correlated selection on and co-evolution of these traits (Sinervo & Svensson, 2002; Pigliucci & Preston, 2004; Bond, 2007). Morphological traits that enhance crypsis, for example, might function to deter predators best (or only) when accompanied by stereotyped behaviour (e.g. 'shaking' of leaf/stick mimics; Skelhorn *et al.*, 2010) or, more commonly, reduced movement (Ruxton *et al.*, 2004; Cooper *et al.*, 2008; Ioannou & Krause, 2009). The suite of selective pressures shaping anti-predator morphology that requires avoiding attention can further stem from trade-offs governing potentially conspicuous behaviours, such as foraging and sexual display (Endler, 1980; Ryan *et al.*, 1982). At another extreme is aposematism, a phenomenon in which predators are deterred by the combination of defenses (e.g. morphological, chemical) and conspicuous and/or memorable traits that advertise unprofitability (Ruxton *et al.*, 2007). This anti-predator strategy might instead weaken any selection imposed by predation costs of finding and procuring food and mates (Lima & Dill, 1990; Speed *et al.*, 2010; Rudh *et al.*, 2012). These cascading trade-offs influence processes as diverse as individual risk assessment (Briffa & Twyman, 2011), diversification and speciation (Santos *et al.*, 2014; Arbuckle & Speed, 2015), and interactions among trophic levels (Ripple & Beschta, 2004).

Understanding whether and how selection acts in concert on anti-predator strategies and other components of fitness is especially tractable when individuals, populations or closely related species fall at different places on the continuum of crypsis to aposematism (Speed *et al.*, 2010; Tarvin *et al.*, 2017). Visual conspicuousness and anti-predator chemical defenses vary considerably both within and among species of poison frogs (Dendrobatidae), with putative crypsis and putative aposematism both evolving multiple times (Caldwell, 1996; Summers

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& Clough, 2001; Santos & Canatella, 2011; Rojas, 2017). A role for colour in deterring attack by local predators is supported, if not unambiguously, by experimental evidence (reviewed by Rojas, 2017). Colour has also been implicated in assortative mate choice (Summers *et al.*, 1999; Maan & Cummings, 2008; Twomey *et al.*, 2014; Gade *et al.*, 2016; Yang *et al.*, 2016, 2019) and male–male communication (Crothers & Cummings, 2013; Yang *et al.*, 2018). The features of colour associated with an advantage in intraspecific contexts may also attract the attention of predators (Maan & Cummings, 2008; Crothers & Cummings, 2013; Dreher *et al.*, 2015), but whether and the extent to which these multiple effects result in a trade-off presumably depends on where the individual/population/species falls on the crypsis to aposematism spectrum.

Measuring the fitness consequences of relationships among visual signals, anti-predator defense and the behaviours they are hypothesized to co-evolve/be co-expressed with (e.g. foraging, courtship) is challenging in poison frogs because observations of predation or even predator attacks on frogs are vanishingly rare (reviewed by Santos & Canatella, 2011; Rojas, 2017). Modelling predator visual systems can suggest which phenotypes might attract or avoid notice (Maan & Cummings, 2012; Willink *et al.*, 2013), and artificial frogs (e.g. clay) can be used to test the relationship between coloration/patterning and attack (at least by the subset of predators that respond to these models; Paluh *et al.*, 2014; Rojas, 2017). Another way to test the hypothesis that diverse coloration and defense in polytypic species reflect different anti-predator strategies (with widespread correlates) is to test the prediction that putatively cryptic populations ought to behave in a way that avoids predator attention and putatively aposematic ones ought to behave as if they are insensitive to this risk (Rudh *et al.*, 2011, 2012; Willink *et al.*, 2014a; Dugas *et al.*, 2015).

We assessed experimentally whether colour is associated with microhabitat preference and a conspicuous behaviour in colour morphs of the polytypic strawberry poison frog, *Oophaga pumilio* (Schmidt, 1857). Native to Central America, *O. pumilio* displays a relatively conserved red body with blue/black limb phenotype across mainland populations (Hagemann & Pröhl, 2007). However, colour morphs spanning the visual spectrum exist along a putative crypsis–aposematism axis in Panama's Bocas del Toro archipelago, with each island or geographical region typically home to a single morph (Siddiqi *et al.*, 2004; Rudh, 2013). Chemical defenses are similarly variable and, overall, are positively associated with visual conspicuousness (Saporito *et al.*, 2006; Maan & Cummings, 2012). Among-population comparisons suggest that males in more conspicuous/well-defended populations spend more time foraging (Pröhl & Ostrowski, 2011) and are more aggressive and explorative than their cryptic counterparts (Rudh *et al.*, 2013). Male *O. pumilio* defend small territories, in which they choose perches from which to call to and search visually for females (Pröhl & Hödl, 1999; Pröhl, 2003; Meuche *et al.*, 2013), an activity that presumably also carries the risk of attracting predators (Ryan *et al.*, 1982).

In their natural habitats, male *O. pumilio* from conspicuous populations use more visually conspicuous perches (Rudh *et al.*, 2011; for similar patterns in the congener *Oophaga granulifera*, see Willink *et al.*, 2013, 2014a). This finding is potentially important to understanding how correlated selection has shaped the evolution of these phenotypes and perhaps even to how this suite of traits might shape reproductive isolation among phenotypically distinct lineages (Rudh *et al.*, 2011; Willink *et al.*, 2013, 2014a). A male's perch in the wild, however, is likely to reflect a compromise between preference, among-site differences in perch availability, competition with other males, and other biotic and abiotic costs associated with perches (e.g. thermal stress) (Rudh *et al.*, 2011; Dugas *et al.*, 2015). Laboratory assays can reveal mate preferences not manifested in choice in the wild (Yang *et al.*, 2019); likewise, assays of display site preference are crucial to testing for co-evolved colour and light habitat preference.

We allowed *O. pumilio* males from three monomorphic populations and three morphs from one polymorphic site to choose between microhabitats with different levels of ambient light availability. Although both the intensity of ambient light and its spectral composition (i.e. colour) vary in ways that influence frog conspicuousness in natural habitats (Endler, 1990, 1993), we manipulated intensity independently of colour because: (1) more intense ambient light should increase the conspicuousness of all *O. pumilio* morphs in a similar way (Maan & Cummings, 2012), and this feature of visual signals mediates detectability by receivers across contexts (e.g. Jones & Osorio, 2004; Cole & Endler, 2015); and (2) habitats used by putatively conspicuous and cryptic morphs in the wild differ in this parameter (Rudh *et al.*, 2011; Willink *et al.*, 2013). Given that aposematism is effective when individuals are conspicuous and crypsis is effective when individuals are inconspicuous, we predicted that the most conspicuous males would spend the most time in high ambient light conditions and spend the most time displaying while in these conditions.

MATERIAL AND METHODS

STUDY POPULATIONS

We studied wild-caught males from four populations in the Bocas del Toro region of Panama (Fig. 1): (1) a monomorphic blue population from the Aguacate peninsula on the mainland (09°10′37.9″N, 82°16′00.4″W); (2) a monomorphic red population from Isla San Cristobal (9°15′50.1″N, 82°15′56.0″W); (3) a monomorphic orange population from Isla Solarte (09°19′16.3″N, 82°29′49.5″W); and (4) a red/blue/intermediate population from the mainland of Panama (9°13′15.70″N, 82°13′5.60″W). The three monomorphic populations span the previously reported range of visual conspicuousness in *O. pumilio* morphs, with orange frogs from Isla Solarte among the most conspicuous, blue frogs from the Aguacate peninsula the least conspicuous, and red frogs from Isla San Cristobal of intermediate conspicuousness (Fig. 1; Pröhl & Ostrowski, 2011; Rudh *et al.*, 2011; Maan & Cummings, 2012). In a red–blue polymorphic region on the Aguacate peninsula, red frogs are more conspicuous than blue ones, with intermediate frogs being, as expected, intermediate (Dugas *et al.*, 2015; Yang *et al.*, 2019). Following previous experimental work, we selected a non-random sample of frogs from the polymorphic population, choosing individuals from the extremes of red and blue and the most 'intermediate' (Yang *et al.*, 2016); by-eye colour classification is equivalent to other methods, at least at these extremes (Dugas *et al.*, 2015; Yang *et al.*, 2016, 2019). We used 20 frogs from each monomorphic population and 23 red, 23 blue and 26 intermediate frogs from the polymorphic population.

STUDY ANIMALS AND EXPERIMENTAL DESIGN

After capture, animals were maintained in captivity at the Smithsonian Tropical Research Institute's Bocas del Toro field station for 7.7 ± 4.9 days (mean ± SD) before testing. During this time, frogs were held in plastic enclosures (37 cm × 22 cm × 25 cm) at low densities (five or fewer frogs per tank). We held tanks outdoors in ambient temperature and humidity; we misted tanks as needed and provided small cups of rainwater to prevent water stress. Frogs consumed wild insects (mostly *Drosophila* spp.) attracted to ripe fruit placed within the tank, and we supplemented this diet with termites. There was no mortality during this study, and we returned all individuals to their place of capture at the end of the experiment.

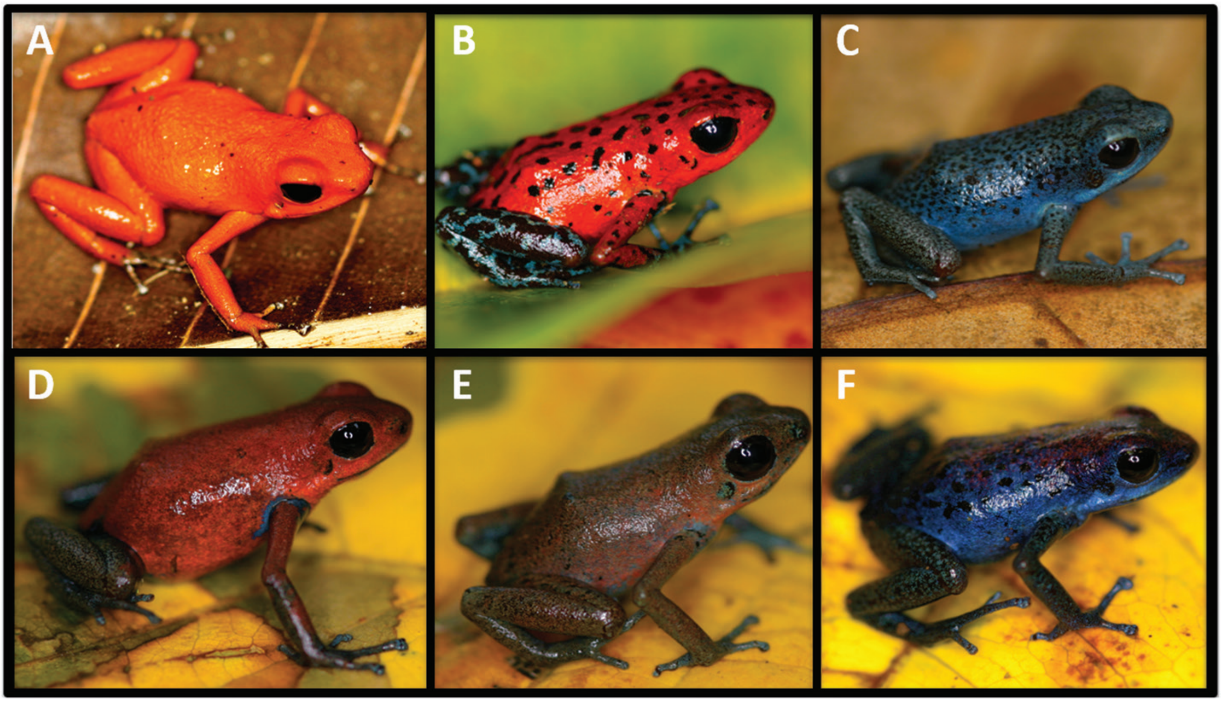

Figure 1. Coloration of *Oophaga pumilio* study populations of from the Bocas del Toro region of Panama. Frogs from Isla Solarte (A) are the most visually conspicuous, with those from Isla San Cristobal (B) intermediate and those from the Aguacate peninsula (C) among the least conspicuous. In a polymorphic population on the Aguacate peninsula, red frogs (D) are more conspicuous than intermediate (E) and blue (F) frogs.

In the late afternoon on the day before an experimental trial, we moved individual frogs from these maintenance tanks to experimental arenas. On a single observation day, we observed either frogs from monomorphic populations (in equal numbers) or frogs from the polymorphic population (equal numbers of red, blue and intermediate individuals). Experimental arenas consisted of three transparent plastic containers (23 cm × 23 cm × 13 cm; hereafter, 'modules') separated by opaque polyvinyl chloride 'tunnels' measuring 5.08 cm in diameter and ~5 cm in length (the minimal distance needed to connect modules; Supporting Information, Fig. S1). We covered the floor of the entire arena in leaf litter that we moistened with ultraviolet-filtered water between trials, and used brown cardboard spacers to prevent visual contact between males in different arenas. In the two modules at either end, we added a small cup filled with water and a small-diameter (~1.5 cm) locally collected stick as a perch. The middle module, in which most ambient light was filtered out (see next paragraph), was intended to function in the same way as a neutral zone would in a dichotomous mate choice assay (e.g. Yang *et al.*, 2016); therefore, a perch and water dish were not provided. When introducing frogs to the arena, we always placed them in the middle (dark, perch-free) module.

We manipulated the ambient light conditions in each module by using theatrical light filters as the roof. These filters reduced the overall intensity of light equally across the light spectrum (i.e. they changed the intensity but not the colour of light). A 'dark' filter (Lee Filters #211) used in the middle (neutral) module allowed 10% light transmittance, a 'light' filter (Lee Filters #130) allowed 95% transmittance, and a 'medium' filter (Lee Filters #20) allowed 70% transmittance. All arenas were kept in a well-covered outdoor area (pilot results suggested that arenas were not robust to the elements). Because ambient light was thus low, we provided supplemental light to arenas with overhead lamps filtered to resemble light conditions at the forest floor (*sensu* Maan & Cummings, 2008; Richards-Zawacki & Cummings, 2011). We conducted all behavioural observations between 21 December 2012 and 4 January 2013. We turned on lights at dawn and began focal observations 30 min later. We recorded male behaviours during four 15 min observations, spaced evenly between our start and 12.00 h, the period of the day during which *O. pumilio* are most active (Graves, 1999).

During behavioural sampling, we recorded the total time spent in each module and the total time spent on elevated perches (the perch, water dish and lip of the polyvinyl chloride tunnel that connected modules). Calling was infrequent; therefore, we did not record or analyse this behaviour. The 119 males (of 132 total) that spent at least some time perching in the light or medium modules spent 41 ± 33% of perched time using the perch, 27 ± 28% using the water dish and 32 ± 33% using the tunnel lip. We summed data for the entire trial (total = 900 s of observation) for subsequent analysis. For four observations (two Aguacate, one each polymorphic red and blue), we did not record the entire observation period; we excluded these samples from total time allocation analyses, but retained them when the dependent variable was a proportion (see 'Statistical analyses').

STATISTICAL ANALYSES

We compared the behaviour of monomorphic populations and of red/blue/intermediate individuals from the polymorphic population with separate analyses. We assessed the relationship between frog colour and preference for microhabitats of various absolute irradiance intensities with two complementary approaches. First, we compared the total time spent outside the dark module among populations (or phenotypes) using a general linear model, with population (or phenotype) as the sole fixed effect; in this analysis, we square-root transformed 'time' to meet the assumption of residual normality. Second, we compared time spent in the high vs. medium light modules (both of which had perches and water dishes) by individuals from different populations (or phenotypes). We used a linear mixed model, with light treatment, population (or phenotype) and the light treatment × population (or phenotype) interaction as fixed effects, and individual as a random effect; degrees of freedom were estimated with the Kenward–Roger approximation. Residuals from this model were normally distributed without transforming the dependent variable 'time' (in seconds).

To ask whether ambient light treatments influenced perching time, we used a generalized linear mixed model with events/trials syntax, in which time spent perching in a lighting environment and total time spent in that environment were treated as events and trials, respectively. The model included ambient light treatment (high or medium), population (or phenotype) and the light × population interaction as fixed effects, and individual as a random effect; we did not include perching behaviour in the dark treatment (intended as a neutral zone) because this module did not contain perches or water dishes (see 'Study animals and experimental design' above). Treating the response variable as events/trials required that instances in which an individual spent no time in a particular module (i.e. trials = 0) were not included in the analysis.

Thus, an individual was represented in the data set one or two times, depending on how many light treatments it used during the experiment. Initially, we used the number of seconds as the unit for our response variable, but these models were overdispersed (χ^2/d.f. for among-population comparison = 131.2, for among-phenotype in the polymorphic population = 172.6). Fit was greatly improved by simply converting the unit to 4 min (χ^2/d.f. for among-population comparison = 1.0, for among-phenotype = 1.3), and we present results from analyses in these units hereafter. We again used the Kenward–Roger approximation for fixed effect degrees of freedom. We used SAS v.9.4 (SAS Institute, Cary, NC, USA) for all analyses.

RESULTS

Allocation of time among modules differing in ambient light

Individuals from all three monomorphic populations spent similar amounts of time in the dark module ($F_{2,55}$ = 1.08, P = 0.346), as did individuals of all three colour types from the polymorphic population ($F_{2,67}$ = 0.12, P = 0.887). Likewise, there was no evidence that differently coloured populations of frogs spent different amounts of time in high or medium light ($F_{1,102}$ = 0.42, P = 0.521) or that the light treatment × population interaction was important ($F_{1,102}$ = 0.80, P = 0.454). In the polymorphic population, frogs did not spend more time in medium than high light ($F_{1,120}$ = 2.57, P = 0.112), and all phenotypes responded in a similar manner (light × phenotype interaction: $F_{2,120}$ = 1.07, P = 0.345). Neither the main effect of population ($F_{2,102}$ = 0.35, P = 0.705) nor phenotype ($F_{2,120}$ = 1.17, P = 0.315) was significant, and stepwise removal of non-significant terms did not have a qualitative effect on the patterns presented above.

Perching behaviour in modules differing in ambient light

In the among-population comparison of time spent in medium and high ambient light, the predicted population × ambient light treatment effect was absent ($F_{2,102}$ = 0.44, P = 0.644). In a model with this interaction removed, there was a marginal effect of ambient light treatment ($F_{1,104}$ = 3.29, P = 0.072), with males spending relatively more time perched in the high light treatment. Populations differed in overall perching behaviour ($F_{2,51.52}$ = 3.93, P = 0.026), with the most cryptic blue frogs perching the most, followed by intermediate red frogs, and conspicuous orange frogs perching the least (Fig. 2).

In the comparison of time spent in medium and high ambient light by red, blue and intermediate frogs from the polytypic population, there was also no evidence that differently coloured individuals perched differently in high and medium light treatment modules (population × ambient light: $F_{2,120}$ = 1.51, P = 0.226; Fig. 2). A model with this interaction removed revealed no overall effect of ambient light on perching ($F_{1,122}$ = 1.23, P = 0.270) but did suggest differences among phenotypes ($F_{2,61.4}$ = 3.38, P = 0.041), with blue and intermediate frogs spending less time perching than red individuals (Fig. 2).

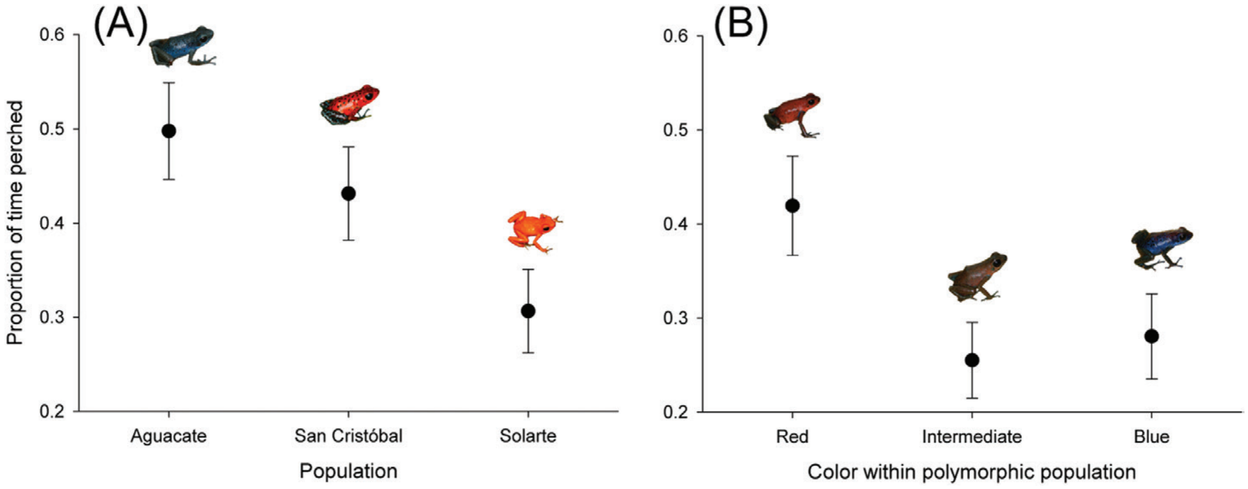

Figure 2. Estimated marginal mean of time spent perching by *Oophaga pumilio* morphs in the ambient light choice experiment. Estimated marginal means are from a model that contained the fixed effects of light (95 or 70% transmittance), which was non-significant, and population (A) or phenotype within a polymorphic population (B).

DISCUSSION

We allowed diversely coloured *O. pumilio* morphs to choose between microhabitats that differed only in the intensity of ambient light, a feature of the environment central to overall signal conspicuousness and crucial to habitat selection in poison frogs (Rudh *et al.*, 2011; Maan & Cummings, 2012; Willink *et al.*, 2013) and other colourful tropical animals (Endler & Théry, 1996; Hill *et al.*, 2001; Heindl & Winkler, 2003). Contrary to predictions drawn from previous work, we found no evidence for microhabitat preference among populations or among phenotypically distinct groups of individuals from a polytypic population hypothesized to span cryptic–aposematic strategies. We found little to suggest that male *O. pumilio* adjusted their perching behaviour in response to ambient light, but populations and phenotypes within the polymorphic population did differ in their overall tendency to spend time on elevated perches. Among populations it was, surprisingly, the least conspicuous males that spent the most time perching. Within a polymorphic population, however, it was indeed the more conspicuous red individuals that spent more time perching than the more cryptic blue or intermediate males. These patterns suggest integrated phenotypes associated with coloration in the polytypic Bocas del Toro *O. pumilio*, but leave open the questions of why among-population differences did not match our predictions and why the relationship between colour and behaviour might not be the same among and within populations.

In the wild, the perches used by male *O. pumilio* of putatively aposematic morphs make frogs easier for predators to see (at least in part because ambient light levels are high) than do perches used by males of putatively cryptic morphs (Rudh *et al.*, 2011; see also Willink *et al.*, 2013, 2014a). Our results suggest, however, that this pattern might not stem exclusively from morph-specific preferences for microhabitats that differ in ambient light intensity. The crucial question remains, then, whether the patterns in nature result from correlated selection on colour and preferences for this feature of microhabitat.

Natural perches can shape signaller fitness via effects of ambient light intensity and spectral composition (i.e. colour) on how receivers detect and assess visual signals (e.g. Endler & Théry, 1996; Rojas *et al.*, 2014b). Given that light intensity and colour covary in habitats used by poison frogs (Endler, 1993), frogs could choose bright habitats by using (and evolving preferences for) criteria other than brightness. Plant material that partly conceals a calling male (Willink *et al.*, 2013) will, for example, alter both the colour and the intensity of ambient light (Endler, 1993). Natural perches also vary in ways that might be independent of light environment. Within *O. pumilio* populations, the height of a perch is important to male reproductive success (Pröhl & Hödl, 1999), and the response to simulated predator approach suggests that high perches are perceived as more valuable (Dugas *et al.*, 2015). Preferences for height and other as yet unexplored features of perches (e.g. acoustic properties; Muñoz & Penna, 2016) could exaggerate or mask any associations between frog colour and light environment preference. This is especially problematic when comparing monomorphic populations, because doing so assumes equal microhabitat availability across habitats (Dugas *et al.*, 2015). Testing this latter assumption might also reveal the role of such differences in habitat in driving and maintaining colour diversity (Marchetti, 1993).

Opposite relationships between visual conspicuousness and time spent perching among and within *O. pumilio* populations might offer further insights into how best to test the hypothesis that anti-predator morphologies are integrated with behavioural traits in polytypic/polymorphic species. If coloration and behaviours become correlated, in full or in part, via plastic responses to actual risk (Lima & Dill, 1990; Stankowich & Blumstein, 2005), among-population differences are difficult to interpret without knowledge of this risk. Perching male frogs might be particularly susceptible to risk, because they call frequently (Pröhl & Hödl, 1999; Pröhl, 2003; Meuche *et al.*, 2013), and their fairly static position while calling might make aposematic coloration less effective (Paluh *et al.*, 2014; Rojas *et al.*, 2014a; Blanchette *et al.*, 2017). Quantification of chemical defense from wild frogs provides some evidence that males might be attacked more often than females (Saporito *et al.*, 2010), meaning that opportunities for actual risk to trigger plastic behavioural responses do exist. Within the red/blue/intermediate polymorphic population, where the background rate of attack risk is presumably the same for males of all colours, we found that the most conspicuous males (the red ones) did spend the most time perching, as predicted if their perceived level of risk was lowest. This pattern suggests that red might be a better defensive colour than blue or intermediate (Hegna *et al.*, 2013; Richards-Zawacki *et al.*, 2013) in this population (as it might be more generally; e.g. Casas-Cardona *et al.*, 2018). Given that perching males are expected to balance the costs and benefits of this activity, it is also possible that the benefits accrued through perching are higher for red males. Even in qualitatively monomorphic populations, there is considerable variation in poison frog coloration, including variation between the sexes (Crothers *et al.*, 2011; Rojas & Endler, 2013), and this variation might be key to uncovering the evolutionary and plastic correlates of anti-predator strategies.

Expectations of how colour and behavioural traits are selected and should co-evolve in groups like poison frogs are based largely on the presumption that evolution occurs along a continuous crypsis–aposematism axis (e.g. Rudh *et al.*, 2011, 2013; Maan & Cummings, 2012; Dugas *et al.*, 2015), a presumption that may warrant further scrutiny. Although the red ubiquitous in mainland *O. pumilio* populations offers protection against predators (Saporito *et al.*, 2007; Paluh *et al.*, 2014), there is scant evidence that local coloration protects against attack in polytypic Bocas del Toro populations (Hegna *et al.*, 2013; Richards-Zawacki *et al.*, 2013; Dreher *et al.*, 2015). This pattern is not conclusive; model prey fool only part of the predator community (Rojas, 2017), and anthropogenic habitat modification has probably reduced the abundance and diversity of the predators with which defenses co-evolved (Summers *et al.*, 2003; Pröhl & Ostrowski, 2011; Richards-Zawacki *et al.*, 2013). Furthermore, the relationship between colour and chemical defense is not perfect (Saporito *et al.*, 2006; Maan & Cummings, 2012). Although the blue (Aguacate) and red (San Cristobal) morphs we studied differ in visual conspicuousness, and behavioural differences have been treated as representatives of different strategies (Rudh *et al.*, 2011, 2012; Rudh, 2013), their chemical defenses might be fairly similar (Maan & Cummings, 2012). The ubiquity of amphibians that are colourful but not chemically defended or chemically defended but not colourful (Wells, 2007) provides further, and strong, evidence that coloration and chemical defense can be subject to independent selective pressures.

Predation can drive the evolution and maintenance of diversity in prey traits (Allen, 1988; Ruxton *et al.*, 2004; Bond, 2007). Studies of such diversity in prey can offer insights into how multiple traits that shape predation risk (e.g. coloration and behaviour; Rojas *et al.*, 2014a) act in conjunction to shape fitness, and these insights can, in turn, be used to generate predictions about how selection should operate on trait combinations in this context, and more broadly (Sinervo & Svensson, 2002; Pigliucci & Preston, 2004; Bond, 2007). When the prediction of integrated expression of traits is met, this confirmation of the underlying hypothesis points to widespread implications for how evolution works (Bond, 2007; Forsman *et al.*, 2008). Equally valuable are cases in which these expectations are not met (e.g. Calsbeek & Cox, 2012), because they suggest even more diverse ways that selection can shape traits with competing and complementary effects on fitness.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Figure S1. Apparatus used to test for ambient light preferences and light-dependent behaviour in *Oophaga pumilio* males. Modules were fitted with lids that allowed 95 (pictured left), 10 (middle) or 70% (pictured right) light transmittance from above. The middle module served as a 'neutral zone', and water dishes and wooden perches were supplied in the other modules to encourage perching display behaviours.