Author/s:
Roberts, SM;Stuart-Fox, D;Medina, I

Title:
The evolution of conspicuousness in frogs: When to signal toxicity?

Date:
2022-11-01

Citation:
Roberts, S. M., Stuart-Fox, D. & Medina, I. (2022). The evolution of conspicuousness in frogs: When to signal toxicity?. Journal of Evolutionary Biology, 35 (11), pp.1455-1464. https://doi.org/10.1111/jeb.14092.

Persistent Link:
https://hdl.handle.net/11343/320041

Received: 7 March 2022 | Revised: 23 June 2022 | Accepted: 19 July 2022
DOI: 10.1111/jeb.14092

JOURNAL OF Evolutionary Biology eseb European Society for Evolutionary Biology

RESEARCH ARTICLE

# The evolution of conspicuousness in frogs: When to signal toxicity?

**Sophie May Roberts** | **Devi Stuart-Fox** | **Iliana Medina**

School of BioSciences, University of Melbourne, Parkville, Victoria, Australia

**Correspondence**
Iliana Medina, School of BioSciences, University of Melbourne, Parkville, Vic. 3010, Australia.
Email: iliana.medina@unimelb.edu.au

**Funding information**
Australian Research Council, Grant/Award Number: DE200100500 and FT180100216

## Abstract

Many organisms use conspicuous colour patterns to advertise their toxicity or unpalatability, a strategy known as aposematism. Despite the recognized benefits of this anti-predator tactic, not all chemically defended species exhibit warning coloration. Here, we use a comparative approach to investigate which factors predict the evolution of conspicuousness in frogs, a group in which conspicuous coloration and toxicity have evolved multiple times. We extracted colour information from dorsal and ventral photos of 594 frog species for which chemical defence information was available. Our results show that chemically defended and diurnal species have higher internal chromatic contrast, both ventrally and dorsally, than chemically undefended and/or nocturnal species. Among species that are chemically defended, conspicuous coloration is more likely to occur if species are diurnal. Our results also suggest that the evolution of conspicuous colour is more likely to occur in chemically defended prey with smaller body size. We discuss potential explanations for this association and suggest that prey profitability (related to body size) could be an important force driving the macroevolution of warning signals.

KEYWORDS
aposematism, defenses, frogs, macroevolution, warning signals

## 1 | INTRODUCTION

Aposematism can be defined as the advertisement of toxicity or unprofitability to a potential predator and is typically achieved through conspicuous coloration or patterning (Ruxton et al., 2004). Warning signals have been shown to facilitate predator learning and lead to dietary conservatism in predators (Gamberale-Stille & Tullberg, 1999; Leimar et al., 1986; Lindström, 1999; Marples et al., 1998); the distinctiveness of warning signals may improve detection by experienced predators and thus reduce recognition errors or forgetting (Guilford, 1990). Recent meta-analyses have shown, across different taxa, that there is a positive relationship between overall conspicuousness and the strength of chemical defences (i.e. level of toxicity), supporting the idea that aposematism is a quantitatively honest signal (White & Umbers, 2021). Not all chemically defended organisms, however, advertise their toxicity; while the presence of conspicuous colours might be an honest signal, the absence of conspicuous colours does not indicate lack of chemical defences. For instance, Ithomiinae butterflies are transparent despite being highly unpalatable (McClure et al., 2019); chemically defended shield bugs from the family Acanthosomatidae are cryptic (Endler & Mappes, 2004); and pufferfish have not evolved conspicuous coloration to advertise one of the most potent toxins in nature (Arakawa et al., 2010). Under what conditions do chemically defended prey evolve conspicuous colours? It remains unclear to what extent the presence or absence of chemical defences can predict the evolution of conspicuous colour features, and which traits can predict the evolution of conspicuous colours in organisms that are already chemically defended.

Perhaps the most expected variable to predict the presence of conspicuous coloration in chemically defended species is diurnal activity. Diurnal species are more commonly at risk from visually hunting predators and, in active species, effective camouflage might be harder to achieve if there is day-time illumination, favouring instead the evolution of warning signals (Merilaita & Tullberg, 2005; Ruxton et al., 2004). In the conspicuous clade of poison-dart frogs (Dendrobatidae), it is thought that a switch to diurnal activity facilitated the exploitation of novel dietary sources, which could have led to increased toxicity and further selection for aposematic coloration (Santos et al., 2003). In other lineages (e.g. Bufonidae) that contain aposematic species, it is also thought that shifts to diurnal activity occurred early on, preceding the evolution of aposematism (Santos & Grant, 2011). In species with chemical defences, colour signals are expected to target visually oriented predators, although colour signals could be involved in sexual selection to some extent as well (Maan & Cummings, 2008). Therefore, we would expect selection to favour the evolution of conspicuous coloration in diurnal and chemically defended species, but not in species that are nocturnal or that are not chemically defended.

Multiple experimental and comparative studies have also suggested a positive relationship between body size and conspicuous coloration. Body size may enhance an aposematic signal or, alternatively, when an animal is already aposematic there might be no selection from predators for it to remain small, leading to a higher prevalence of aposematism in larger prey (Ruxton et al., 2004; Smith et al., 2014). In insects, dendrobatid frogs and salamanders, for example, there is a positive association between body size and anti-predator signalling or conspicuousness (Hagman & Forsman, 2003; Loeffler-Henry et al., 2019; Medina et al., 2020; Winebarger et al., 2018). On the other hand, some studies suggest that a positive relationship between body size and conspicuousness is not favoured if a prey is already conspicuous due to its large size. Tseng et al. (2014), for instance, showed that body size in weevils may already be used as a warning signal in the absence of aposematic coloration. Selection for conspicuous coloration could also be influenced by prey profitability, whereby when a prey is highly profitable, predators are more willing to ingest toxin in exchange for the nutritional benefits (Skelhorn et al., 2016; Smith et al., 2014). Under this scenario, the benefits of advertising toxicity with conspicuous colours may be lower in larger prey. Given the variation in results across studies, and the restricted phylogenetic scope in many of these, we still lack an understanding of the link between aposematism and body size at a broad evolutionary scale.

Anurans represent an ideal clade to study the broad scale evolution of aposematic coloration (Rojas, 2017; Rudh & Qvarnström, 2013). Warning signals have been extremely well studied in poison dart frogs (Dendrobatidae; Maan & Cummings, 2012; Santos et al., 2016; Summers & Clough, 2001), but toxicity and aposematism are also common in other anuran families such as Bufonidae, Myobatrachidae and Mantellidae (Saporito et al., 2012; Vences et al., 2003). Capitalizing on the multiple independent origins of aposematism in frogs, we use a comparative framework to study its evolution and investigate (1) to what extent different aspects of conspicuous coloration are associated with the presence of chemical defences in frogs and (2) which species traits (body size, diurnal activity) predict conspicuousness in frogs that are chemically defended.

## 2 | METHODS

### 2.1 | Photograph collection

We collected photographs of dorsal and ventral views for anuran species (one per species per view, dorsal: 594 spp., ventral: 445 spp.) which had previously had their chemical defence status established (Arbuckle & Speed, 2015). Most photos were obtained from websites such as inaturalist.com or calphotos.com or google image search (Supplementary Material). To ensure that species had not been mislabeled, we checked that general colours matched across the different views, and confirmed identity using AmphibiaWeb (https://amphibiaweb.org). We selected photos of the highest quality that were not over or under exposed and minimized variation in view (i.e. were taken as close as possible from a perpendicular view to dorsal or ventral). Although the photos are unstandardized, they provide biologically meaningful colour information for broad-scale comparative analyses (Kang et al., 2017; Loeffler-Henry et al., 2019; Medina et al., 2020). Photos or drawings from field guides can represent well the colouration of a species and should offer comparable results to standardized measures when used across species (Dale et al., 2015; Medina et al., 2020). In our case, photos were the best available resource given that frog colour is not retained in museum specimens and collecting standardized photos for hundreds of live frog species is not feasible. However, we recogise the caveats of using non-stardadised images, which ignore the UV component of signals and could in some circumstances exaggerate the contrast between colours. This is why, in addition to data extracted from photographs, we also use a previously published classification of frog coloration into "conspicuous" and "not conspicuous" from Arbuckle and Speed (2015).

### 2.2 | Colour extraction

Images were analysed using the software Image J (Schneider et al., 2012). From each photo, we manually extracted pixel intensity values for the red (R), green (G) and blue (B) channels for each distinct colour observed (from one up to a maximum five colours) on the dorsal surface of the frog (all extraction done by SR). As photos were not standardized, we did not use an automatic colour extraction and analysis software; instead, we chose specific regions in the photograph to ensure that they were free of lighting artefacts (no specular highlights, not in shadow) and that clearly represented each distinct colour within the frog outline. Our preliminary analyses indicated that this manual approach more reliably identified the primary colour patches and their colour values than an automated approach for images that varied in angle of view and illumination. Colours were classified into one of three categories according to the area they occupied within the frog's outline: >30%, between 30% and 10%, and less than 10% of the area. We recorded which colour patches were adjacent to each other and to the background (i.e. were present at the edge of the frog's outline and thus adjacent to the background, from a dorsal view). We extracted colour information for 60 backgrounds from the same photographs, containing either green (leaves, grass) or brown (trunks, ground) coloration. These values were used as samples of natural background colours, following (Medina et al., 2017), details are provided in the Supplementary Material.

Using RGB values from photos, we estimated both internal and background contrast, because both measures can be important in the efficacy of a warning signal (Aronsson & Gamberale-Stille, 2009; Prudic et al., 2006). Internal contrast was calculated as the contrast between the two dominant, adjacent colours within the frog's outline, and background contrast was calculated as the contrast of the dominant colour adjacent to the natural background against both average green and brown backgrounds. For each of these components (internal and background contrast), we calculated both contrast in colour (chromatic contrast) and contrast in brightness (luminance contrast). Colour and luminance are known to facilitate predator aversion, with luminance contrast being potentially more relevant for colourblind predators (Prudic et al., 2006). Chromatic contrast was calculated as the Euclidean distance in a two-dimensional colour space where axes are the standardized difference between red and green ((R − G)/(R + G + B)) and green and blue ((G − B)/(R + G + B)) channels (Endler, 1990; Grill & Rush, 2000).

$$\text{Chromatic contrast} = \sqrt{\left[\left(\frac{R1-G1}{R1+G1+B1}\right)-\left(\frac{R2-G2}{R2+G2+B2}\right)\right]^2+\left[\left(\frac{G1-B1}{R1+G1+B1}\right)-\left(\frac{G2-B2}{R2+G2+B2}\right)\right]^2}$$

Luminance contrast was calculated as the difference between the average RGB value (luminance) of adjacent colour patches.

$$\text{Luminance contrast} = \left(\left(R_1+G_1+B_1\right)/3\right)-\left(\left(R_2+G_2+B_2\right)/3\right)$$

where subscripts denote the two colour patches. Full details of contrast calculations are given in the Supplementary Material. These measures are independent of a viewer's visual system, but highly correlated with perceived conspicuousness to tetrachromatic predators such as birds (Smith et al., 2016). In total, we used eight variables to describe dorsal and ventral conspicuousness: six contrast measures for dorsal coloration (internal chromatic and luminance contrast; chromatic and luminance contrast against green and brown backgrounds) and two for ventral coloration (internal chromatic and luminance contrast). These variables were not highly correlated with each other (most $r^2 < 0.5$, Figure S2), so they were used independently as response variables in subsequent analyses.

## 2.3 | Additional variables: toxicity, body size and activity time

We used information on toxicity data from Arbuckle and Speed (2015). This data set classified species into two categories (chemically defended or not) based on a range of different published sources. Data on nocturnal and diurnal activity of frog species was obtained mainly from a global database of ecological traits (Oliveira et al., 2017) in which activity during the day or night was recorded independently as "yes" or "no". From this information, we created a new variable with three levels: nocturnal, diurnal and "no data". Species that exhibited both nocturnal and diurnal activity were scored as diurnal, since we were interested in the presence of activity during the day. As additional sources we also extracted information from Anderson and Wiens (2017) and Callaghan and Rowley (2021). For the first one, we collated information on whether a species was diurnal/nocturnal or arrhythmic. If these were 'arrhythmic' we reclassified them as diurnal, given they could be found also during the day. Callaghan and Rowley (2021) published information on the 'percentage of diurnality' of a species, based on the number of calls that were recorded during the day relative to the total number of calls recorded. We considered a species as diurnal or nocturnal if more than 90% of the time they were recorded calling during the day or night, respectively. We highlight that these classifications only reflect available evidence; for example, if a species has been recorded as nocturnal (but not diurnal), it does not mean that it is not diurnal, it means that so far there is no evidence of diurnal activity. Lastly, we also extracted information on overall species size (SVL, mm) from Womack and Bell (2020).

## 2.4 | Statistical analyses

### 2.4.1 | Association between conspicuous coloration and chemical defence

For all analyses, we used the PGLS function (phylogenetic generalized least squares) in the R package CAPER 1.0.1 (Orme et al., 2018) in R 4.0.3 (RStudio-Team, 2020) and used maximum likelihood to estimate the phylogenetic signal (lambda) of each model. To test the link between chemical defence and conspicuousness, we ran models with each of the eight contrast measures as the response variable and a predictor variable with four categories (defended/diurnal, defended/nocturnal, undefended/diurnal, undefended/nocturnal). We used this approach rather than a model with two predictor variables (defence and time of activity) because there was an association between diurnal activity and toxicity, and diurnal species were more likely to be toxic. We also ran models that only included a binary variable of chemical defence (defended/undefended) because not all species had information on time of activity, so the sample size is larger ($n = 455$ spp. vs. 594 spp.).

### 2.4.2 | Predictors of conspicuousness in chemically defended species

In a second set of models, we performed PGLS analyses to test which variables were the best predictors of conspicuousness in chemically defended frog species ($n = 370$ spp). We ran models with each of the eight contrast measures as the response variable and log(body size) and time of activity (diurnal, nocturnal and 'no information') as predictors. Results were qualitatively identical when including or excluding species with no information. We included interactions between time of activity and body size, since we expected body size to be associated with conspicuousness in diurnal but not nocturnal species. Using the Arbuckle and Speed (2015) data set, we also tested whether species classified as conspicuous versus inconspicuous differed in body size. This colour classification in independent from our colour data, so this analysis offers an additional source of evidence for the patterns presented. Since conspicuousness was classified as a binary variable in this data set, we used the R package phylolm (Ho et al., 2016) and a phylogenetically controlled logistic model with conspicuousness (1/0) as the response variable and log body size as the predictor.

Finally, we examined whether the use of either dorsal or ventral conspicuous coloration for aposematic signalling was associated with body size. To do this, we created a new variable by calculating the difference between dorsal and ventral internal chromatic contrast (with larger values representing more contrasting dorsal coloration). We did not necessarily expect a linear relationship between body size and this new variable, because small values could be present in species that are either cryptic or contrasting on both sides. Therefore, we grouped species into four categories based on the difference between dorsal and ventral internal chromatic contrast, and overall contrast: species with higher dorsal contrast, higher ventral contrast, high contrast on both sides and low contrast on both sides. Categories were created based on the distribution of the continuous variable described before (difference between dorsal and ventral chromatic contrast), and details are shown in Supplementary Material (Figure S1). We then used a PGLS to test whether there were differences in body size (response variable) between species that signal dorsally, ventrally or on both sides.

In all analyses, we accounted for phylogenetic relationships by using a published time calibrated consensus tree (Jetz & Pyron, 2018). When significant patterns were detected, we tested the effect of phylogenetic uncertainty by repeating the analyses using 100 additional trees taken from the posterior distribution of a Bayesian phylogenetic analysis (Jetz & Pyron, 2018). For all models, we report estimates, *t*-values and *p*-values. We used the R packages GGPLOT2 (Wickham, 2016) and GGTREE (Yu, 2020) for all figures.

## 3 | RESULTS

### 3.1 | Association between conspicuous coloration and chemical defence

Of the 594 frog species for which we extracted RGB values, 370 were chemically defended whilst 224 lacked chemical defences (Figure 1). Colour variables extracted were correlated to some extent, but most correlations were moderate ($r^2 < 0.50$, Figure S2). Six out of eight colour variables were strongly linked to the presence of chemical defences (dorsal internal chromatic contrast, ventral chromatic and luminance contrast, luminance contrast against green and chromatic and luminance contrast against brown, Figure 4). Across all species, those that were diurnal and chemically defended had higher internal chromatic contrast (both dorsally and ventrally; Tables 1 and S4) and higher dorsal chromatic contrast against brown backgrounds (Figure 2; Tables S1 and S4). For luminance contrast, ventral internal contrast and dorsal contrast against brown and green were all predicted by the presence of chemical defences and diurnal activity (Table 1); whereas dorsally, there was no relationship between chemical defences and internal luminance contrast.

### 3.2 | Predictors of conspicuousness in chemically defended species

For chemically defended species, both dorsal and ventral internal chromatic contrast were higher in smaller species, if these were diurnal (Figure 3). This was true when using the whole data set that included species with no data on time of activity (Figure 2; Tables S2 and S5) and also when using a reduced data set including only species with complete data (Tables S6 and S7). Diurnal species were significantly smaller ($F = 6.851$, $p$-value $= 0.001$), but this did not cause multicollinearity issues in our models, as all VIF (variance inflation factors) were below 2. In any case, models including only body size as predictor showed the same association with colour (Table S3). We found strong negative associations between body size and multiple colour variables in species that are chemically defended. We did not find an association between body size and conspicuousness in species that are not chemically defended (Figure 3, Table S3). We also found negative associations between body size and ventral internal luminance contrast and dorsal luminance contrast against the background (Tables S2 and S5). Associations between body size and conspicuousness were also evident when using Arbuckle and Speed's (2015) binary measure of conspicuousness; models using phylogenetic control and conspicuousness as a binary response variable showed a significant link between smaller body size and conspicuousness, only in diurnal species (diurnal, $B = -0.16$, $t$-value $= -2.21$, $p = 0.028$; nocturnal $B = -0.032$, $t$-value $= -0.66$, $p = 0.509$; Figure 3A).

There was no significant association between body size and categories of chemically defended species with only dorsal or ventral

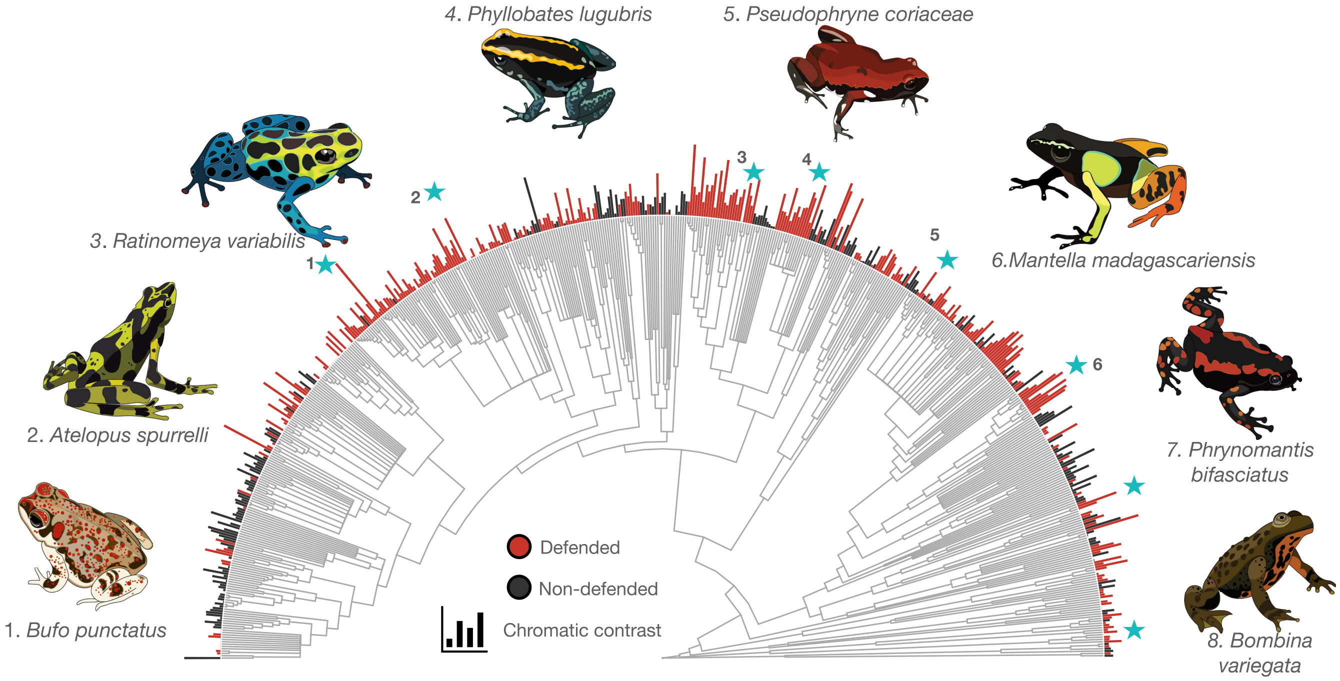

FIGURE 1 Phylogenetic tree showing species included in the study (594 spp.) and information on chemical defences (red vs. grey) and internal chromatic contrast (bar length). We note that the prevalence of chemical defences in this sample of species is high, and possibly a result of biases in searching for defences in species that are already suspected to have those. Illustrations by Daniela Perez

TABLE 1 Model results (PGLS) for association between chemical defences, diurnal activity and internal chromatic and luminance contrast (dorsal and ventral). Results presented using consensus tree

| | Dorsal | | | Ventral | | |
|---|---|---|---|---|---|---|
| | Estimate | *t*-value | *p*-value | Estimate | *t*-value | *p*-value |
| Internal contrast (chromatic) | | | | | | |
| Defended diurnal versus defended nocturnal | −0.072 | −4.090 | <0.001 | −0.055 | −3.116 | 0.002 |
| Defended diurnal versus not defended diurnal | −0.084 | −4.012 | <0.001 | −0.070 | −3.388 | 0.001 |
| Defended diurnal versus not defended nocturnal | −0.065 | −3.183 | 0.002 | −0.051 | −2.428 | 0.016 |
| Chemically defended versus not defended | 0.033 | 2.681 | 0.007 | 0.028 | 2.163 | 0.031 |
| Internal contrast (luminance) | | | | | | |
| Defended diurnal versus defended nocturnal | −7.869 | −1.282 | 0.201 | −18.007 | −2.229 | 0.026 |
| Defended diurnal versus not defended diurnal | −13.441 | −1.845 | 0.066 | −26.203 | −2.778 | 0.006 |
| Defended diurnal versus not defended nocturnal | −0.867 | −0.122 | 0.903 | −32.486 | −3.389 | 0.001 |
| Chemically defended versus not defended | 2.070 | 0.472 | 0.636 | 16.540 | 2.735 | 0.006 |

*Note*: Estimates from analysis on MCC tree, results across 100 trees reported in Supplementary Material.

contrasting colouration (Figures 4a and S4, Estimate = 0.151, *t*-value = 1.653, *p*-value = 0.101). However, sample size was small for some categories in this analysis, due to lack of species signalling exclusively dorsally or ventrally (26 spp. with only dorsal signal and 14 spp. with only ventral signal).

For all analyses with significant patterns, these were consistent when the analyses were performed across 100 trees (Tables S4–S7). A graphic summary of main results can be seen in Figure 4. We did not adjust the significance level due to multiple testing using Bonferroni correction because this procedure is highly conservative and can obscure biologically important patterns (Nakagawa, 2004), but in any case, the main associations reported would remain significant after such correction (new level $p < 0.006$).

## 4 | DISCUSSION

Conspicuousness in toxic species often serves as an anti-predator warning signal, yet the global evolutionary drivers of these signals remain poorly understood (Kikuchi et al., 2021). We evaluated whether factors such as chemical defence, diurnal activity, and body size could

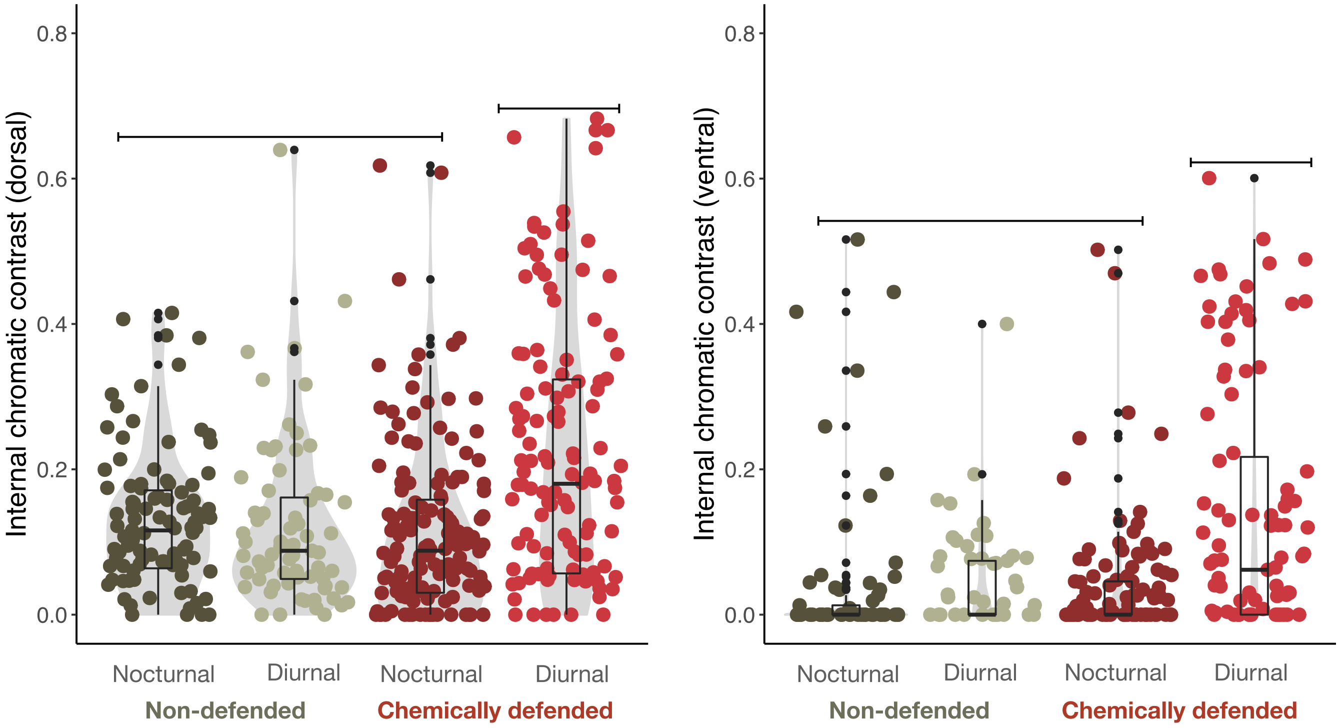

**FIGURE 2** Differences in internal chromatic contrast according to defence status and time of activity, for dorsal (left) and ventral (right) views. Lines on top highlight groups with significant differences

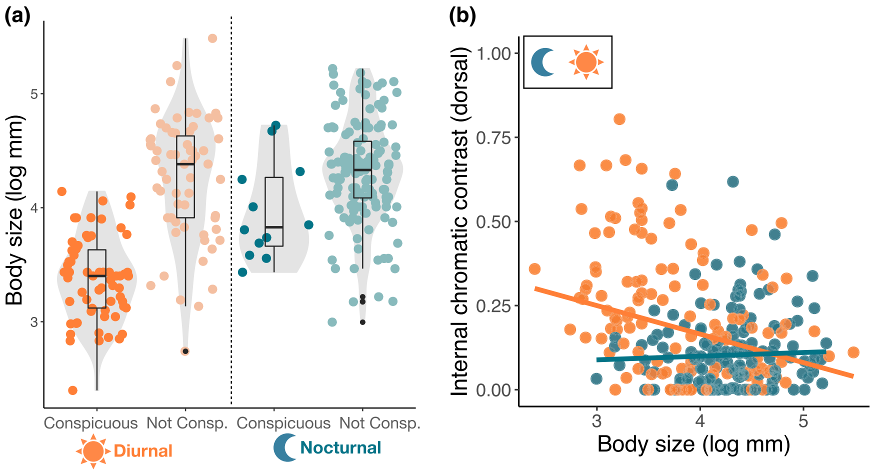

**FIGURE 3** Association between body size and internal chromatic contrast for diurnal (orange) and nocturnal species (blue). (a) When using binary colour classification from Arbuckle and Speed (2015). (b) When using continuous measures of conspicuousness extracted in this study. All species included in these figures are chemically defended. Slopes in (b) were estimated from phylogenetically controlled models (Table S2)

predict conspicuous coloration in frogs. We found that chemically defended species that are diurnal have greater chromatic contrast between colour pattern elements, both dorsally and ventrally, and against brown backgrounds. They also have greater luminance contrast between ventral colour pattern elements. In addition, we found that toxic species are more conspicuous when they are diurnal and have a smaller body size. Together, these results support at a broader scale the role of colour contrast as a warning signal in frogs and reveal that the association between body size and conspicuousness could be more complex than previously proposed. Although it has been shown that large frogs are more likely to be aposematic (Hagman & Forsman, 2003; Santos & Cannatella, 2011), our findings indicate that when species are toxic, the smaller ones are more conspicuous.

Our analyses suggest that the chromatic component of the internal colour (i.e. hue, saturation) is more strongly linked to aposematic signalling than luminance, at least dorsally, which is consistent with broad observations in other clades (Lawrence & Noonan, 2018; Ruxton et al., 2004). Chromatic contrast can be more important than luminance contrast for object recognition in birds, especially when targets are large (Zylinski & Osorio, 2013). Chromatic signals provide robust information under variable illumination conditions and are stable and strong even when light conditions are low (Arenas et al., 2014); colour associations are also more efficiently learned than achromatic associations, which could be key in the evolution of warning signals (Kazemi et al., 2014; Osorio et al., 1999; Stevens, 2007). However, the lack of association between chemical

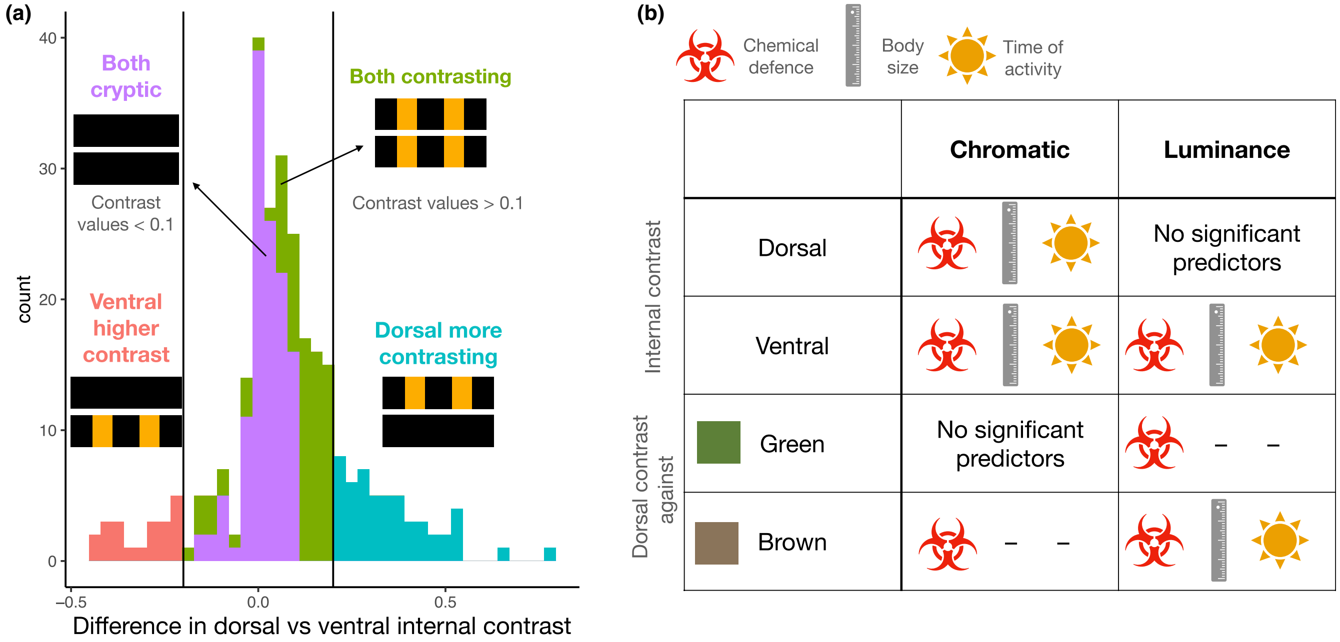

FIGURE 4 (a) Distribution of ventral versus dorsal internal contrast, with number of species in each category. (b) Graphic summary of results for all the colour variables extracted, icons represent significant links between each predictor (chemical defence, body size and diurnal activity) and the response colour variable. Models testing links with chemical defence included the complete data set, and models testing links between body size and time of activity included only chemically defended species (since we were interested in predictors of conspicuousness in these species)

defence and internal contrast in luminance can also be expected for other reasons, besides predator cognition. High internal luminance contrast can be found in both conspicuous and camouflaged colour patters, especially in the case of disruptive coloration (Schaefer & Stobbe, 2006). For instance, cryptic colour patterns comprising black and light grey or brown patches will have high internal luminance contrast (Stevens & Merilaita, 2009). Given the closer correspondence between conspicuousness and chromatic than luminance contrast, we would expect a stronger association between chemical defence and the former, and this expectation was corroborated by our results.

Our analyses also revealed that variation in *internal contrast* across species can be better explained by time of activity and body size than variation in contrast against backgrounds. Most internal contrast variables were linked to the presence of chemical defences and, in toxic frogs, to body size and time of activity. This was not the case for contrast against green, and only partially for contrast against brown backgrounds. We acknowledge that this could be product of our approach, where using one colour measure to represent green and brown natural backgrounds could limit our power to explain variation in background contrast. Likewise, differences in microhabitat use could affect background contrast, given that some species might be mostly arboreal and commonly found on green backgrounds while other species might be on the ground most of the time. Nevertheless, the approach of using average brown and green backgrounds has been able to explain colour evolution in other systems (Delhey et al., 2010, 2013; Medina et al., 2017). It has been shown experimentally that chicks learn faster to avoid prey when it contrasts against the background, but not when there is a high internal contrast (Aronsson & Gamberale-Stille, 2009). However, internal contrast is independent of variation in background coloration; thereby ensuring conspicuousness against any background. For active or mobile species that may encounter a variety of backgrounds, internal contrast may provide a more consistent and reliable signal; and indeed is a feature of most warning coloration (Barnett et al., 2016; Stevens & Ruxton, 2012).

Ventral coloration is often involved in deimatic displays (Umbers et al., 2017) and could, potentially, have an important function in intraspecific communication (Maan & Cummings, 2008; Wang & Shaffer, 2008). Our results show a strong link between ventral internal luminance contrast and chemical defence. It is unclear, however, why some chemically defended species signal ventrally instead of dorsally, and this does not seem to be related to body size (although species signalling only ventrally tend to be smaller). One possibility is that dorsal colour may be under conflicting selection for being distinctive/recognizable but not necessarily highly detectable, as some level of camouflage may be useful, especially in weakly defended species. Ventral colour, however, can be more conspicuous as it is hidden from view and does not affect detection. For species with only ventral signalling (e.g. *Bombina*, *Melanophryniscus*), camouflage may be the main defence, with warning colours as an alternative defence, once the prey is detected (Toledo et al., 2011). In our data set, however, having only a conspicuous ventral signal was less common than having only a dorsal one.

For chemically defended species, we found that diurnal and smaller species were more likely to evolve conspicuous coloration. Warning signals that involve colour to advertise toxicity are thought to be directed towards visually oriented predators, such as birds or lizards (Ratcliffe & Nydam, 2008; Ruxton et al., 2004). To our knowledge, this is the first time a link between diurnal activity and conspicuousness has been reported across frogs, but this association has been shown in other lineages. In lepidoptera, in seven phylogenetically matched pairs of clades, aposematic colour evolved in the diurnal but not in the nocturnal clades (Merilaita & Tullberg, 2005). It is unclear whether changes from nocturnal to diurnal behaviours preceded the evolution of toxicity and conspicuousness in frogs, but in Dendrobatidae and Bufonidae (two of the families with the highest number of aposematic species) diurnal activity is suggested to be ancestral, and to have preceded the evolution of toxicity, which was then followed by the evolution of conspicuous coloration in chemically defended species (Grant et al., 2006; Santos & Grant, 2011).

Previous studies have found a positive association between conspicuousness and body size in frogs. These studies, however, included both defended and undefended species. Therefore, these studies suggest that aposematic species are larger than species that are non-toxic and cryptic, but they do not provide information on whether size can predict the evolution of conspicuousness in chemically defended frogs. In our data set, chemically defended species tended to be larger than non-defended species, broadly supporting the idea that larger species are better defended; however, among species that are toxic, those that are large are less likely to be conspicuous (in both chromatic and luminance contrast). Our results match previous findings in nudibranchs, where larger species were more likely to be cryptic (Cheney et al., 2014). Most nudibranchs are chemically defended, which means that, similar to our results, chemically defended nudibranchs that are smaller are more likely to be conspicuous.

A negative link between body size and conspicuousness could arise because the increased profitability of toxic prey (in this case their large size) could reduce selection for conspicuous signals, and instead favour crypsis to avoid detection. It has been shown experimentally that when relative profitability is high, then crypsis is a better alternative than aposematism (Johansen et al., 2011). Intake of toxic prey increases when the nutritional content is artificially increased, suggesting that the nutritional value of prey can impact the evolution of anti-predator strategies (Halpin et al., 2013; Skelhorn et al., 2016; Smith et al., 2014). Moreover, many frog toxins are produced in the skin, leading to a lower surface area/volume ratio and lower relatively toxicity in larger frogs (Santos et al., 2016). Body size may be negatively associated with conspicuous coloration in toxic frogs for other reasons too. For example, if size serves as a memorable signal in itself, as demonstrated by Tseng et al. (2014) in weevils, this may reduce the need for conspicuousness in larger species, although some studies have shown that colour is a more salient feature than size (Halpin et al., 2013). In addition, larger animals generally contain more toxin and may, therefore, be less palatable and already better defended (Jeckel et al., 2019). Interestingly, it has been suggested that ingesting toxic, but profitable prey, could allow investment in detoxification processes and can favour the evolution of mechanisms to overcome toxicity in predators (Halpin et al., 2013). If this is the case, then predator–prey coevolution related to toxicity could be more common in larger defended species, where their high relative profitability increases the probability that predators ingest toxic prey. This, in turn, relaxes selection for warning signals in larger prey because these signals are less likely to deter predators.

Our study has shown that chromatic aspects of colour patterns and internal contrast are strongly related to the evolution of chemical defences in frogs. When to signal toxicity? We have established that frog species signal their toxicity when they are active during the day and have a smaller body size, and these two variables could help to explain the prevalence of warning signals in different communities. Future studies could explore the extent to which these macroevolutionary patterns apply to other aposematic clades, marine and terrestrial, and the precise mechanisms driving the negative link between body size and conspicuousness.

## ACKNOWLEDGEMENTS

We would like to thank Ben Phillips and Mark Elgar for insightful comments on earlier versions of this study. Thank you also to every member of the Stuart-Fox Lab, for the fascinating weekly zooms, laughs and input.

## FUNDING INFORMATION

Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians. IM and DSF would like to thank the Australian Reserch Council for funding (DE200100500 and FT180100216).

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/jeb.14092.

## DATA AVAILABILITY STATEMENT

Data set is available in Dryad at https://doi.org/10.5061/dryad.hmgqnk9kz.

## ORCID

*Iliana Medina* https://orcid.org/0000-0002-1021-5035

## REFERENCES

Anderson, S. R., & Wiens, J. J. (2017). Out of the dark: 350 million years of conservation and evolution in diel activity patterns in vertebrates. *Evolution*, *71*, 1944–1959.

Arakawa, O., Hwang, D.-F., Taniyama, S., & Takatani, T. (2010). Toxins of pufferfish that cause human intoxications. *Coastal Environmental and Ecosystem Issues of the East China Sea*, *227*, 244.

Arbuckle, K., & Speed, M. P. (2015). Antipredator defenses predict diversification rates. *Proceedings of the National Academy of Sciences*, *112*, 13597–13602.

Arenas, L. M., Troscianko, J., & Stevens, M. (2014). Color contrast and stability as key elements for effective warning signals. *Frontiers in Ecology and Evolution*, *2*, 25.

Aronsson, M., & Gamberale-Stille, G. (2009). Importance of internal pattern contrast and contrast against the background in aposematic signals. *Behavioral Ecology*, *20*, 1356–1362.

Barnett, J. B., Scott-Samuel, N. E., & Cuthill, I. C. (2016). Aposematism: Balancing salience and camouflage. *Biology Letters*, *12*, 20160335.

Callaghan, C. T., & Rowley, J. J. (2021). A continental assessment of diurnality in frog calling behaviour. *Austral Ecology*, *46*, 65–71.

Cheney, K. L., Cortesi, F., How, M. J., Wilson, N. G., Blomberg, S. P., Winters, A. E., Umanzör, S., & Marshall, N. J. (2014). Conspicuous visual signals do not coevolve with increased body size in marine sea slugs. *Journal of Evolutionary Biology*, *27*, 676–687.

Dale, J., Dey, C. J., Delhey, K., Kempenaers, B., & Valcu, M. (2015). The effects of life history and sexual selection on male and female plumage colouration. *Nature*, *527*, 367–370.

Delhey, K., Hall, M., Kingma, S. A., & Peters, A. (2013). Increased conspicuousness can explain the match between visual sensitivities and blue plumage colours in fairy-wrens. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20121771.

Delhey, K., Roberts, M., & Peters, A. (2010). The carotenoid-continuum: Carotenoid-based plumage ranges from conspicuous to cryptic and back again. *BMC Ecology*, *10*, 13.

Endler, J. A. (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society*, *41*, 315–352.

Endler, J. A., & Mappes, J. (2004). Predator mixes and the conspicuousness of aposematic signals. *The American Naturalist*, *163*, 532–547.

Gamberale-Stille, G., & Tullberg, B. (1999). Experienced chicks show biased avoidance of stronger signals: An experiment with natural colour variation in live aposematic prey. *Evolutionary Ecology*, *13*, 579–589.

Grant, T., Frost, D. R., Caldwell, J. P., Gagliardo, R., Haddad, C. F., Kok, P. J., Means, D. B., Noonan, B. P., Schargel, W. E., & Wheeler, W. C. (2006). Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). *Bulletin of the American Museum of Natural History*, *2006*, 1–262.

Grill, C. P., & Rush, V. N. (2000). Analysing spectral data: Comparison and application of two techniques. *Biological Journal of the Linnean Society*, *69*, 121–138.

Guilford, T. (1990). The secrets of aposematism: Unlearned responses to specific colours and patterns. *Trends in Ecology and Evolution*, *5*, 323.

Hagman, M., & Forsman, A. (2003). Correlated evolution of conspicuous coloration and body size in poison frogs (*Dendrobatidae*). *Evolution*, *57*, 2904–2910.

Halpin, C., Skelhorn, J., & Rowe, C. (2013). Predators' decisions to eat defended prey depend on the size of undefended prey. *Animal Behaviour*, *85*, 1315–1321.

Ho, L. S. T., C. Ane, R. Lachlan, K. Tarpinian, R. Feldman, Q. Yu, W. van der Bijl, J. Maspons, R. Vos, and M. L. S. T. Ho (2016). Package 'phylolm'. See http://cran.r-project.org/web/packages/phylolm/index.html. Accessed February 2018.

Jeckel, A. M., Kocheff, S., Saporito, R. A., & Grant, T. (2019). Geographically separated orange and blue populations of the Amazonian poison frog *Adelphobates galactonotus* (Anura, Dendrobatidae) do not differ in alkaloid composition or palatability. *Chemoecology*, *29*, 225–234.

Jetz, W., & Pyron, R. A. (2018). The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nature Ecology & Evolution*, *2*, 850–858.

Johansen, A. I., A. Exnerová, K. H. Svádová, S. Pavel, B. S. Tullberg, and G. Gamberale-Stille (2011). Anti-predatory defences in the shieldbug *Graphosoma lineatum* (Heteroptera: Pentatomidae) differ between life stages and colour forms: An experiment with hand reared great tits (*Parus major*) as predators.

Kang, C., Zahiri, R., & Sherratt, T. N. (2017). Body size affects the evolution of hidden colour signals in moths. *Proceedings of the Royal Society B: Biological Sciences*, *284*, 20171287.

Kazemi, B., Gamberale-Stille, G., Tullberg, B. S., & Leimar, O. (2014). Stimulus salience as an explanation for imperfect mimicry. *Current Biology*, *24*, 965–969.

Kikuchi, D. W., Herberstein, M. E., Barfield, M., Holt, R. D., & Mappes, J. (2021). Why aren't warning signals everywhere? On the prevalence of aposematism and mimicry in communities. *Biological Reviews*, *96*(6), 2446–2460.

Lawrence, J., & Noonan, B. P. (2018). Avian learning favors colorful, not bright, signals. *PLoS One*, *13*, e0194279.

Leimar, O., Enquist, M., & Sillen-Tullberg, B. (1986). Evolutionary stability of aposematic coloration and prey unprofitability: A theoretical analysis. *The American Naturalist*, *128*, 469–490.

Lindström, L. (1999). Experimental approaches to studying the initial evolution of conspicuous aposematic signalling. *Evolutionary Ecology*, *13*, 605–618.

Loeffler-Henry, K., Kang, C., & Sherratt, T. N. (2019). Consistent associations between body size and hidden contrasting color signals across a range of insect Taxa. *The American Naturalist*, *194*, 28–37.

Maan, M. E., & Cummings, M. E. (2008). Female preferences for aposematic signal components in a polymorphic poison frog. *Evolution: International Journal of Organic Evolution*, *62*, 2334–2345.

Maan, M. E., & Cummings, M. E. (2012). Poison frog colors are honest signals of toxicity, particularly for bird predators. *The American Naturalist*, *179*, E1–E14.

Marples, N. M., Roper, T. J., & Harper, D. G. (1998). Responses of wild birds to novel prey: Evidence of dietary conservatism. *Oikos*, *83*, 161–165.

McClure, M., Clerc, C., Desbois, C., Meichanetzoglou, A., Cau, M., Bastin-Héline, L., Bacigalupo, J., Houssin, C., Pinna, C., Nay, B., Llaurens, V., Berthier, S., Andraud, C., Gomez, D., & Elias, M. (2019). Why has transparency evolved in aposematic butterflies? Insights from the largest radiation of aposematic butterflies, the Ithomiini. *Proceedings of the Royal Society B: Biological Sciences*, *286*, 20182769.

Medina, I., Delhey, K., Peters, A., Cain, K. E., Hall, M. L., Mulder, R. A., & Langmore, N. E. (2017). Habitat structure is linked to the evolution of plumage colour in female, but not male, fairy-wrens. *BMC Evolutionary Biology*, *17*, 35.

Medina, I., Vega-Trejo, R., Wallenius, T., Symonds, M. R. E., & Stuart-Fox, D. (2020). From cryptic to colorful: Evolutionary decoupling of larval and adult color in butterflies. *Evolution Letters*, *4*, 34–43.

Merilaita, S., & Tullberg, B. S. (2005). Constrained camouflage facilitates the evolution of conspicuous warning coloration. *Evolution*, *59*, 38–45.

Nakagawa, S. (2004). A farewell to Bonferroni: The problems of low statistical power and publication bias. *Behavioral Ecology*, *15*, 1044–1045.

Oliveira, B. F., São-Pedro, V. A., Santos-Barrera, G., Penone, C., & Costa, G. C. (2017). AmphiBIO, a global database for amphibian ecological traits. *Scientific Data*, *4*, 170123.

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W (2018). CAPER (Comparative Analyses of Phylogenetics and Evolution in R). 1.0.1.

Osorio, D., Miklósi, A., & Gonda, Z. (1999). Visual ecology and perception of coloration patterns by domestic chicks. *Evolutionary Ecology*, *13*, 673–689.

Prudic, K. L., Skemp, A. K., & Papaj, D. R. (2006). Aposematic coloration, luminance contrast, and the benefits of conspicuousness. *Behavioral Ecology*, *18*, 41–46.

Ratcliffe, J. M., & Nydam, M. L. (2008). Multimodal warning signals for a multiple predator world. *Nature*, *455*, 96–99.

Rojas, B. (2017). Behavioural, ecological, and evolutionary aspects of diversity in frog colour patterns. *Biological Reviews*, *92*, 1059–1080.

RStudio-Team. (2020). R version 4.0.3 – "Bunny-Wunnies Freak Out".

Rudh, A., & Qvarnström, A. (2013). Adaptive colouration in amphibians. In *Seminars in cell & developmental biology* (pp. 553–561). Elsevier.

Ruxton, G., T. Sherratt, and M. Speed (2004). *Avoiding attack: The evolutionary ecology of crypsis, warning signals and mimicry*. Oxford University Press.

Santos, J. C., & Cannatella, D. C. (2011). Phenotypic integration emerges from aposematism and scale in poison frogs. *Proceedings of the National Academy of Sciences*, *108*, 6175–6180.

Santos, J. C., Coloma, L. A., & Cannatella, D. C. (2003). Multiple, recurring origins of aposematism and diet specialization in poison frogs. *Proceedings of the National Academy of Sciences*, *100*, 12792–12797.

Santos, R. R., & Grant, T. (2011). Diel pattern of migration in a poisonous toad from Brazil and the evolution of chemical defenses in diurnal amphibians. *Evolutionary Ecology*, *25*, 249–258.

Santos, J. C., Tarvin, R. D., & O'Connell, L. A. (2016). A review of chemical defense in poison frogs (Dendrobatidae): Ecology, pharmacokinetics, and autoresistance. *Chemical Signals in Vertebrates*, *13*, 305–337.

Saporito, R. A., Donnelly, M. A., Spande, T. F., & Garraffo, H. M. (2012). A review of chemical ecology in poison frogs. *Chemoecology*, *22*, 159–168.

Schaefer, H. M., & Stobbe, N. (2006). Disruptive coloration provides camouflage independent of background matching. *Proceedings of the Royal Society B: Biological Sciences*, *273*, 2427–2432.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*, 671–675.

Skelhorn, J., Halpin, C. G., & Rowe, C. (2016). Learning about aposematic prey. *Behavioral Ecology*, *27*, 955–964.

Smith, K. R., Cadena, V., Endler, J. A., Kearney, M. R., Porter, W. P., & Stuart-Fox, D. (2016). Color change for thermoregulation versus camouflage in free-ranging lizards. *The American Naturalist*, *188*, 668–678.

Smith, K. E., Halpin, C. G., & Rowe, C. (2014). Body size matters for aposematic prey during predator aversion learning. *Behavioural Processes*, *109*, 173–179.

Stevens, M. (2007). Predator perception and the interrelation between different forms of protective coloration. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 1457–1464.

Stevens, M., & Merilaita, S. (2009). Defining disruptive coloration and distinguishing its functions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*, 481–488.

Stevens, M., & Ruxton, G. D. (2012). Linking the evolution and form of warning coloration in nature. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 417–426.

Summers, K., & Clough, M. E. (2001). The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 6227–6232.

Toledo, L. F., Sazima, I., & Haddad, C. F. (2011). Behavioural defences of anurans: An overview. *Ethology Ecology & Evolution*, *23*, 1–25.

Tseng, H.-Y., Lin, C.-P., Hsu, J.-Y., Pike, D. A., & Huang, W.-S. (2014). The functional significance of aposematic signals: Geographic variation in the responses of widespread lizard predators to colourful invertebrate prey. *PLoS One*, *9*, e91777.

Umbers, K. D. L., De Bona, S., White, T. E., Lehtonen, J., Mappes, J., & Endler, J. A. (2017). Deimatism: A neglected component of antipredator defence. *Biology Letters*, *13*, 20160936.

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. *Organisms Diversity & Evolution*, *3*, 215–226.

Wang, I. J., & Shaffer, H. B. (2008). Rapid color evolution in an aposematic species: A phylogenetic analysis of color variation in the strikingly polymorphic strawberry poison-dart frog. *Evolution: International Journal of Organic Evolution*, *62*, 2742–2759.

White, T. E., & Umbers, K. D. (2021). Meta-analytic evidence for quantitative honesty in aposematic signals. *Proceedings of the Royal Society B*, *288*, 20210679.

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag.

Winebarger, M. M., Pugh, M. W., Gangloff, M. M., Osbourn, M. S., & Siefferman, L. (2018). Body size is positively correlated with conspicuous coloration in ambystoma salamanders, but negatively correlated with conspicuous coloration in plethodon salamanders. *Frontiers in Ecology and Evolution*, *6*. https://doi.org/10.3389/fevo.2018.00143

Womack, M. C., & Bell, R. C. (2020). Two-hundred million years of anuran body-size evolution in relation to geography, ecology and life history. *Journal of Evolutionary Biology*, *33*, 1417–1432.

Yu, G. (2020). Ggtree: A serialized data object for visualization of phylogenetic tree and annotation data. *Methods in Ecology and Evolution*, *8*(1), 28–36.

Zylinski, S., & Osorio, D. (2013). Visual contrast and color in rapid learning of novel patterns by chicks. *Journal of Experimental Biology*, *216*, 4184–4189.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Roberts, S. M., Stuart-Fox, D., & Medina, I. (2022). The evolution of conspicuousness in frogs: When to signal toxicity? *Journal of Evolutionary Biology*, *00*, 1–10. https://doi.org/10.1111/jeb.14092