ORIGINAL ARTICLE

RAPID POPULATION DIVERGENCE LINKED WITH CO-VARIATION BETWEEN COLORATION AND SEXUAL DISPLAY IN STRAWBERRY POISON FROGS

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The likelihood of speciation is assumed to increase when sexually selected traits diverge together with ecologically important traits. According to sexual selection theory, the evolution of exaggerated display behavior is driven by increased mating success, but limited by natural selection, for example, through predation. However, the evolution of aposematic coloration (i.e., an ecologically important trait) could relieve the evolution of exaggerated display behavior from the bound of predation, resulting in joint divergence in aposematic coloration and sexual display behavior between populations. We tested this idea by examining conspicuousness, using color contrasts between individuals and their native backgrounds, and sexual display of 118 males from genetically diverged populations of the Strawberry poison frog, *Dendrobates pumilio*. Our results show that the level of conspicuousness of the population predicts the sexual display behavior of males. Males from conspicuous populations used more exposed calling sites. We argue that changes in aposematic coloration may rapidly cause not only postmating isolation due to poorly adapted hybrids, but also premating isolation through shifts in mating behaviors.

KEY WORDS: Aposematism, natural selection, *Oophaga pumilio*, population differentiation, sexual selection, speciation.

Ecological divergence caused by natural selection is thought to play a major role in speciation (Darwin 1859; Orr and Smith 1998; Schluter 2001; Coyne and Orr 2004), but this process may often fail to result in the evolution of complete reproductive isolation (Nosil et al. 2009). A central aim in evolutionary biology is therefore to pinpoint the crucial conditions needed for ecological divergence to result in speciation. Given that strict allopatric conditions rarely last throughout the whole speciation process, the homogenizing effect of gene flow has been identified as a major obstacle against the completion of the process (e.g., Mayr 1963;

Felsenstein 1981; Rice and Hostert 1993). Three main conclusions from theoretical modeling are therefore that speciation is promoted by: (1) fast ecological divergence causing strong selection against hybrids, (2) evolution of sexual isolation in parallel with ecological divergence, and (3) a mechanism for maintaining linkage disequilibrium of the genes underlying the traits selected against in hybrids and sexual isolation (reviewed by Servedio 2008). Thus, whether ecological divergence leads to complete speciation may to a large extent depend on the interaction between natural and sexual selection.

The evolution of exaggerated display behaviors or traits is, according to both theory and a vast body of empirical evidence, promoted by sexual selection and constrained by natural selection (Andersson 1994). In wolf spiders, for example, it has been shown that boldness in sexual display both increases male mating success and correlates positively with predation risk (Kotiaho et al. 1998). However, bright coloration or bold display behavior need not always be associated with an increased predation risk. Aposematic species signal to visual predators that they are unpalatable prey by using bright colors (Poulton 1890). They depend on the strength of the signal their bodies send to predators as it increases not only the ease of detection and speed of predator learning (Gittleman and Harvey 1980; Gittleman et al. 1980), but also the strength of the learned aversion (reviewed in Ruxton et al. 2006). Thus, aposematically colored individuals need not hide from predators to ensure their survival. This could allow an increase in other traits such as sexual display behaviors that are otherwise limited by the cost of predation. For cryptic prey, sexual display and mating behavior would be constrained due to the usual trade-off between using a visually and vocally effective call perch and avoiding detection by predators. This would be true even if the cryptic prey were toxic because the strength of the learned

aversion depends on aposematic coloration (reviewed in Ruxton et al. 2006). In aposematic species on the other hand, detection by predators would be less adverse, or possibly even advantageous under the assumption that predators readily treat the species signal/color as a sign of unpalatability. Thus, a relief from predation risk associated with visual detection should benefit bold individuals, which then could gain a mating advantage without paying the cost of increased predation. Such a relationship would mean that the highest individual fitness would be achieved at a relatively low level of exposure during mate attraction and courtship in cryptic species and at a higher level of exposure in aposematic species (Fig. 1). These assumptions are based on the use of different morphologic predation-avoidance strategies (aposematic or cryptic coloration) and not on the exact level of toxicity to the predator. Although the evolution of aposematism requires some degree of toxicity, there need not be a perfect correlation between level of toxicity and level of aposematic coloration.

By combining the well-tested main assumptions underlying the theories of sexual selection and evolution of aposematic coloration, our model (Fig. 1) predicts that the predator avoidance strategy (cryptic vs. aposematic) should coevolve with exaggerated display traits. Such a coevolution would be favorable for the evolution of reproductive isolation between diverging populations. This is because divergence in an ecologically important trait (i.e., predator avoidance strategy) may promote prezygotic isolation as a side effect through a corresponding divergence in sexual display traits.

The island populations of the strawberry poison frog, *Dendrobates pumilio*, provide an excellent system to test the prediction of our model. These frogs are diurnal and mainly aposematically colored. The species inhabits Central American lowlands from Nicaragua in the northwest to central Panama in the southeast
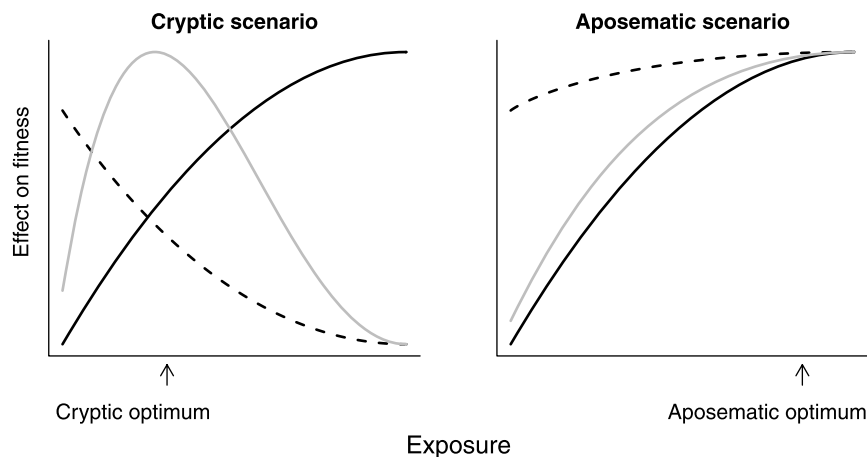

Figure 1. Model on coevolution of sexual display and predation avoidance strategy. Effect on fitness derived from intraspecific effects (black solid) and predation avoidance (black dashed) in a cryptic versus an aposematic prey scenario. Gray lines show these two effects combined visualizing the expected exposure optima during sexual display in aposematic versus cryptic prey.
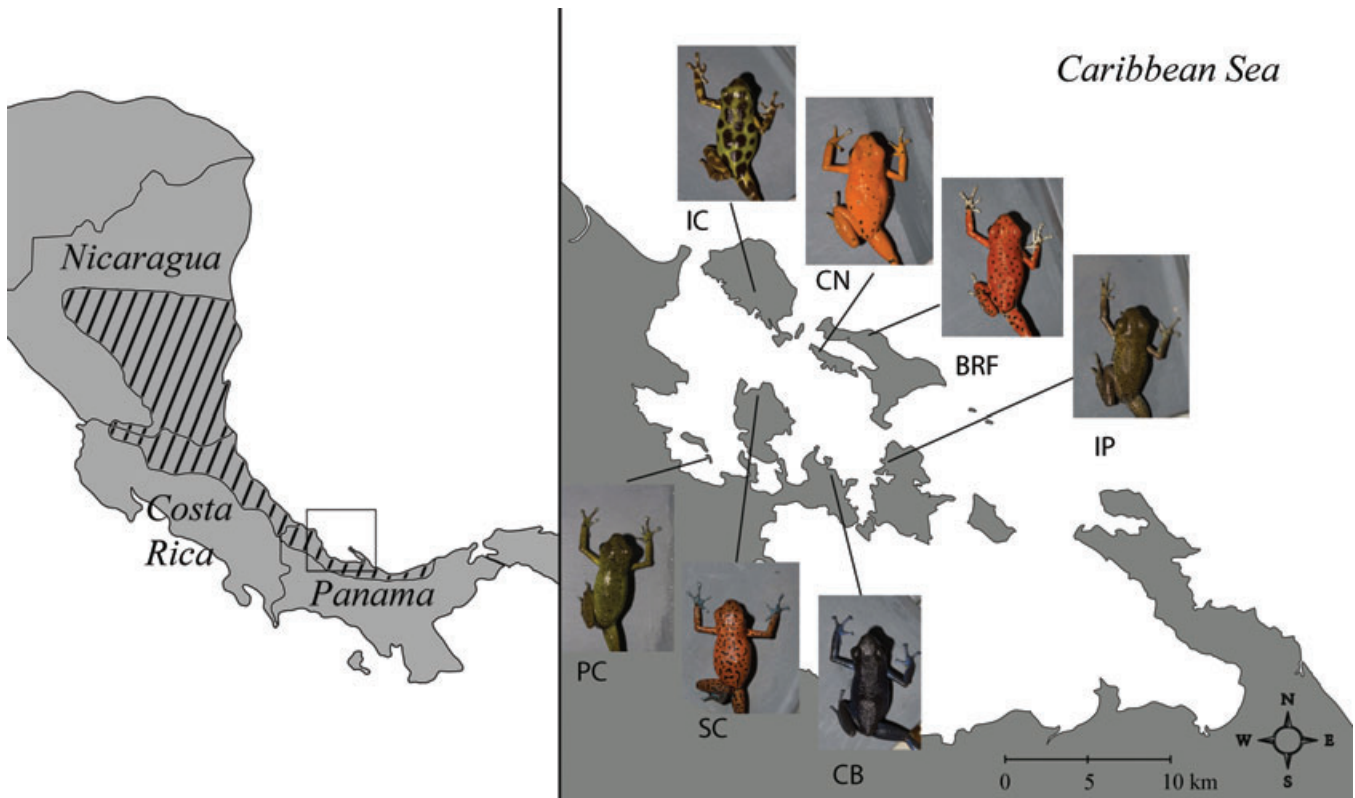
Figure 2. Left: Distribution of *D. pumilio* (striped area) and sample area (box), right: a detailed map of sample area with typical individuals of sampled populations.

(Savage 2002) (Fig. 2) and in most its distribution it is monomorphic with a reddish dorsum and blue/dark legs (Hagemann and Prohl 2007). However, it exhibits great variation in coloration in the southern part of its distribution; in and around the Bocas del Toro archipelago, Panama (Summers et al. 2003; Hagemann and Prohl 2007; Rudh et al. 2007). Although a slight population and sex interaction on color was found in one population by Rudh et al. (2007), and significantly brighter males than females was found in another population by Maan and Cummings (2009), no general sexual dichromatism has been found on either the mainland or the island populations using reflectance data (A. Rudh, unpubl. data). The islands in Bocas del Toro have gradually formed as a result of sea-level rise during the last 10,000 years, some islands becoming isolated as recent as a thousand years ago (Summers et al. 1997; Anderson and Handley 2001, 2002; Wang and Shaffer 2008). Therefore the majority of the color variation among individuals from these populations most likely represents a case of rapid morphological divergence, and thus provides us with a unique system to investigate patterns of trait evolution in nature with few confounding effects.

In *D. pumilio*, males hold territories that are used as call site, courtship area, and oviposition site (Pröhl and Hödl 1999) and there is a strong prior resident effect where resident males usually win fights (Baugh and Forester 1994; Pröhl and Hödl 1999). Male

mating success has been found to correlate with calling activity and average perch height (Pröhl and Hödl 1999), suggesting that exposure promotes mating success. Several studies of the toxicity of populations in this species have been published (e.g., Daly and Myers 1967; Saporito et al. 2004, 2006, 2007a). Daly and Myers (1967) tested for correlations between toxicity and bright coloration but did not find any significant patterns. As mentioned earlier, the only crucial assumption about toxicity in the predicted relationship between coloration and sexual display is that bright colored prey is toxic, and we can conclude that individuals from all investigated populations contain at least some toxic skin alkaloids.

Some aspects of sexual selection, especially female mate preference for their own color morph, have been studied in detail in this system (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008), which would lead to synergetic effects of natural and sexual selection (Maan and Cummings 2009). By contrast, our model only assumes synergetic effects of natural and sexual selection under the aposematic scenario whereas the two forces will work in opposing directions under the cryptic scenario. We focus on a behavioral trait known to influence mating success that is, exposure when calling, and investigate the combined effects of exposure and conspicuousness on the visual signal that both potential predators and mates can use. Thus, the aim of this study is to investigate sexual display behavior in relation to

visual conspicuousness to a potential predator among populations of Strawberry poison frogs. We predict that males with a cryptic predator avoidance strategy (i.e., with a cryptic dorsal coloration) would face a stronger constraint on their sexual behavior by predation risk and therefore choose to display at less-exposed sites than conspicuous males.

Material and Methods

Seven populations from six islands and one peninsula throughout the western part of Bocas del Toro archipelago (Fig. 2) were evaluated for their color contrast and level of male sexual display.

CONSPICUOUSNESS

Based on previous field studies populations were grouped into color contrast groups according to our perception of dorsal coloration (Table 1). The validity of this grouping was later justified by the use of spectral reflectance data collected for individuals and backgrounds during November–December 2007 and May–June 2008. To collect direct measurements of the individuals and backgrounds, we used a teleradiometer that was calibrated following Sumner et al. (2005). The teleradiometer consisted of an Avantes portable spectrophotometer (AvaSpec-2048FT-SPU), connected with a 400-nm optic cable to a modified Nikon FM2 camera with a Nikon Rayfact PF10545MF-UV Quartz lens (Nikon Corporation, Tokyo, Japan) with a quartz filter. A Nikon HS-8 s (Nikon Corporation, Tokyo, Japan) lens shade was attached to the lens that was set for an aperture of 4.5 and a focus distance of 48 cm. Avantes AvaSoft® 7.0 software was used to record and visualize the spectral data. The dorsal coloration was usually measured three times for each individual although a few individuals were only measured once or twice due to fine color patterns for example, speckled individuals. The teleradiometer was calibrated to an Avantes WS-2 white diffuse reflection standard before each individual was measured. Measurements were visually controlled and, when available, the median reflectance spectrum was chosen for further analysis. To allow contrast calculation between each frog's dorsal color and the natural background substrates, we chose nine different background types representing local typ-

ical brown soil, common bark, and live green leafs as well as dead yellow leafs, reddish leafs, grayish leaves and three different types of typical brown colored leaf for our leaf litter estimation. Each background was measured five times and the median measurement was selected to represent that specific background.

Spectra were imported to a purpose written software (Håstad and Ödeen 2008) and color distance (ΔS) (Vorobyev and Osorio 1998; Osorio et al. 2004) between frog dorsal color and the natural background color was calculated on spectra from 320 to 700 nm and interpolated to a step width of 1 nm. *D. pumilio* is diurnal and uses a colorful signal that implies that the signal receivers are also diurnal and have color vision, indicating birds as likely important predators. Further, birds are predators on many tropical anurans (Poulin et al. 2001) and experiments show that birds commonly attack clay models frogs (Noonan and Comeault 2009) and even discriminate between colors as seen by fewer predatory attempts on red compared to brown models (Saporito et al. 2007b). Thus, spectral sensitivity data of *Pavo cristatus* (peafowl) with SWS1 : SWS2 : MWS : LWS cone proportions of 1 : 1.9 : 2.2 : 2.1 was used in the calculation to represent a bird with a typical violet sensitive vision system (Hart 2002). We chose a representative forest light measured at locality CN (Fig. 3, compared with D65 standard light).

Quantum flux was set to 10,000 representing moderately shaded day light condition (Osorio et al. 2004). This results in an absolute measure of perceived difference between two colors in units of just noticeable differences (JNDs). One JND describes the lower limit of chromatic discrimination under ideal conditions (Osorio et al. 2004), although the actual limit under field conditions might very well be higher. The JNDs are determined by the noise in the visual system where the source of the noise and the magnitude depends on light intensity (e.g., Osorio et al. 2004). For a specific situation, the perceived intensity can be calculated from vision parameters of the receiver and measured light levels (Vorobyev 2003).

To obtain ecologically relevant contrast estimations, we used data on backgrounds where frogs were found during the field seasons of 2007 and 2008. In total, 384 observations were classified

Table 1. Sampling localities with abbreviations, color contrast grouping (2 groups and 3 groups), and sample sizes for color contrast estimations and male exposure.

| Locality	Abb.	Two groups	Three groups	n color contrast	n exposure
Bastimentos red frog beach	BRF	Conspicuous	Conspicuous	25	18
Cayo Nancy	CN	Conspicuous	Conspicuous	39	16
Isla Colón	IC	Conspicuous	Intermediate	32	24
Isla San Cristobal	SC	Conspicuous	Intermediate	18	20
Isla Popa	IP	Cryptic	Cryptic	20	12
Pelican Cay	PC	Cryptic	Cryptic	10	15
Cerro Brujo	CB	Cryptic	Cryptic	9	13
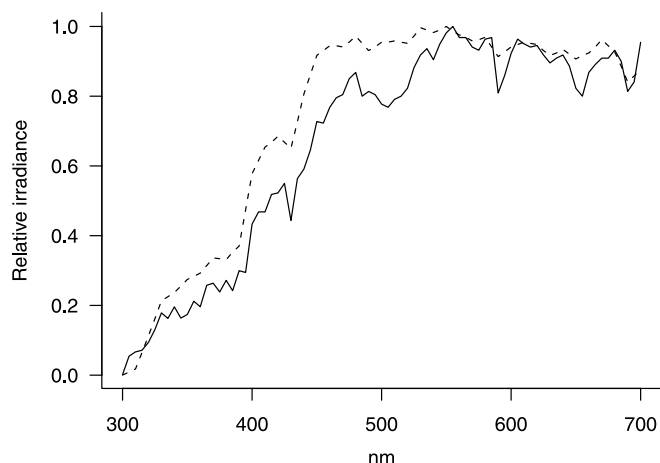
Figure 3. Relative irradiance of forest light used for contrast estimations (solid) compared with D65 standard light (dashed).

as follows: 130 bark, 23 live green leaves, 203 dead leaf litter, and 14 soil. The remaining 14 observations consisted mostly of rocks, which were only found in one locality (not present in this study) and these observations were therefore excluded from further analysis. Leaf litter (54.9%) was divided into the four classes described above and the proportion of these classes was set, by manually scoring digital photographs of leaf litter at the natural habitat, to: yellow leaves 0.04, reddish leaves 0.15, grayish leaves 0.15, and the remaining three brown leaves 0.22 each. These proportions of backgrounds were used to weight the ΔS values proportionally. Frogs from different populations could possibly choose to sit on different types of backgrounds and thereby change their conspicuousness. If, for example, green frogs chose to sit on green leafs they will appear less conspicuous than if they sit on brown leaf litter. Although looking at the observations in the analyzed populations (BRF = 23, CN = 18, IP = 5, SC = 19, CB = 3 IC = 26, PC = 20) we see no such tendencies. The brown backgrounds that give a very similar output from the vision model (bark, soil, and leaf litter) account for 88–100% of the observations in each population and the only population with green leaf observations of more than 4% is SC (11%), which still is in line with our conspicuousness grouping.

Prey detection might not always depend on color vision in birds, because objects also can be distinguished by brightness differences alone. For achromatic tasks and possibly also motion detection, birds are assumed to use their double cones (V. Campenhausen and Kirschfeld 1998; Hart and Hunt 2007). To calculate the achromatic contrast between frog dorsal spectrum and background spectrum, we therefore used the double cone spectral sensitivity of the peafowl (Hart 2002) following Siddiqi et al. (2004).

$$\Delta L = |\Delta f / \omega|,$$

where ΔL is the brightness contrast, Δf is the difference between the focal spectra detected by the double cones and ω is the Weber fraction estimated as 0.05 for the double cone (Siddiqi et al. 2004).

EXPOSURE

Call site exposure data were collected during May to June 2008. Male frogs were located by their call. Once located, all males had to be observed calling to be included in the analysis. The call sites were marked with a flag and measured within 30 min. Incident light was measured in field using an Avantes portable spectrophotometer (AvaSpec-2048FT-SPU, Avantes, Eerbeek, The Netherlands), connected with a 400-nm optic cable to a cosinus corrector. The angle was selected so that the corrector pointed at the most open and light area from where a possible avian predator might observe the frog. The corrector was attached to a tripod to allow a stable placement and correct angle. It also allowed the person recording the measurements not to influence the light. Reference measurements were taken before and after all call site measurements, and when there were changes between clouds and clear sky or uneven cloud cover. All measurements were taken between 1100 and 1300 to standardize the angle and strength of the incident light and to minimize the time effect on behavior (Graves et al. 2005). Three measurements for each call site/reference were visually controlled and the median was selected for further analysis. Measurements were recorded with Avantes AvaSoft 7.0 software, converted and imported to a purpose written software (Håstad and Ödeen 2008). Each spectrum was analyzed between 320 to 700 nm and interpolated to a step width of 1. Spectra were standardized to counts per millisecond, controlled for the equipment used and transformed to $Q/m^2/s/nm$. The total area of the spectrum was calculated and also each call site value (Ac) was divided with its sun reference (As) and the ratio was used as an estimate of relative exposure (Ac/As).

THE EFFECT OF POSSIBLE FINE-SCALE BEHAVIORAL ADJUSTMENTS

We minimized the variation in reference light intensity (As) between individuals and populations by collecting measurements at the same time of day and only on days with either clear skies or light cloud cover. Although the remaining variation is small and color contrast groups do not show a significant difference (Table 2) we need to consider a possible fine-scale individual behavioral adjustment and include this in a second analysis. The presence or absence of such an adjustment determines the preferred way of estimating exposure.

If there is not a behavioral response, a male is assumed to display at the same call site independent of change in cloud cover. This would result in a constant and comparable relative exposure

Table 2. ANOVA table of linear mixed effect model (LME) for analysis of relative exposure (*Ac/As*), absolute exposure (*Ac*), and sun reference (*As*).

	Num df	Den df	*F*-value	*P*-value
$(Ac/As)^{0.25}$				
Two groups	1	5	10.210	0.024
Three groups	2	4	10.983	0.024
$Ac^{0.25}$				
Two groups	1	5	9.186	0.029
Three groups	2	4	3.835	0.118
As				
Two groups	1	5	0.533	0.498
Three groups	2	4	1.421	0.342

value for each male. In other words, call site light intensity (*Ac*) changes proportionally to changes in sun reference light intensity (*As*)

$$\text{Relative exposure} = Ac/As.$$

However, there may be a fine-scale individual behavioral adjustment to changes in cloud cover. If a male would choose a relatively more shaded display site at more clear skies and vice versa, to maintain a constant absolute exposure, it would lead to relative exposure values that are inconsistent. This is caused by the fact that call site light intensity (*Ac*) is kept consistent (by the behavioral adjustment) but sun reference intensity (*As*) changes. Hence, assuming a fine-scale behavioral adjustment, an absolute exposure value is preferred over a relative exposure value to obtain accurate results.

$$\text{Absolute exposure} = Ac$$

STATISTICAL ANALYSIS

All statistical analyses were performed using R (R-Development-Core-Team 2008). To group populations into color contrast groups, we tested if population means in color contrast to the background were higher than the mean background color variation (the mean of all pairwise color contrasts of background substrate spectra) with one sample, one sided *t*-tests. The effect of color contrast group on male exposure was analyzed using linear mixed-effects models (LME) where population was implemented as a random effect nested under color contrast group. The LMEs were fitted using restricted maximum likelihood (REML). In order not to violate the LME assumptions regarding heteroscedasticity between analytical units and residuals distributions, exposure was fourth root transformed. Inclusion of the explanatory fixed effect (color contrast group) and inclusion of a third (intermediate) color contrast group was validated using log likelihood tests (Crawley 2007) as described below. For the test of the hypothesis that more

conspicuous males show higher sexual display, we performed analysis of variance (ANOVA) as implemented in nlme (Pinheiro et al. 2008). The assumption of statistical independence of populations in the LME model could be violated by phylogenetic patterns. Because all populations were monophyletic with generally high support (Wang and Shaffer 2008) and have very low estimated migration rates (Rudh et al. 2007; Wang and Shaffer 2008; Wang and Summers 2010), we analyzed the relationship between color contrast group and male exposure with a phylogenetic generalized least squares (PGLS) model. A previously published ML phylogeny was obtained from Wang and Shaffer (2008). Pelican cay was not included in this phylogeny, however, due to close morphologic resemblance and geographic proximity to Isla Pastores (Summers et al. 2003, here called Shepherd Island) it was used to represent Pelican cay in the phylogeny. We restricted the phylogeny to the populations in this study and used it to create a phylogenetic correlation structure (Martins and Hansen 1997) obtained with package ape (Paradis et al. 2004). We then used a generalized linear model in package nlme (Pinheiro et al. 2008) including the obtained correlation structure and fitted by REML.

Results
CONSPICUOUSNESS

The grouping of populations into color groups based on our perception was subsequently validated based on their ΔS values. To get an estimate of background color variation, the nine background measurements were contrasted to each other following previously described calculations and settings resulting in 36 pairwise ΔS values. On average, individuals from populations IP, PC, and CB showed values close to 4 whereas individuals from populations BRF, CN, IC, and SC all showed ΔS values above that of the mean background color distance variation (mean 5.13 ± 0.53 SE, Fig. 4). None of the cryptic populations showed a higher color contrast than the mean pairwise background color distance (IP: $t_{19} = -4.59$, $P = 1$, PC: $t_9 = -1.40$, $P = 0.90$ and CB: $t_8 = -2.40$, $P = 0.98$) whereas all conspicuous populations clearly did (BRF: $t_{24} = 26.27$, $P < 0.001$, CN: $t_{38} = 23.13$, $P < 0.001$, IC: $t_{31} = 4.93$, $P < 0.001$ and SC: $t_{17} = 4.30$, $P < 0.001$).

The leaf litter composition could probably change noticeably between seasons and possibly change the contrast estimations. However, testing an array of different proportions resulted in only small changes in ΔS values and a consistent division using our background reference method even with a 100% increase or decrease in the brightly colored red and yellow leaves. This also shows that a potential bias from our visual scoring of leaf litter proportions does not affect the results. ΔL values generally followed the pattern seen in ΔS (Fig. 4). However for CB a relatively higher mean was obtained (Fig. 4). This result of population CB
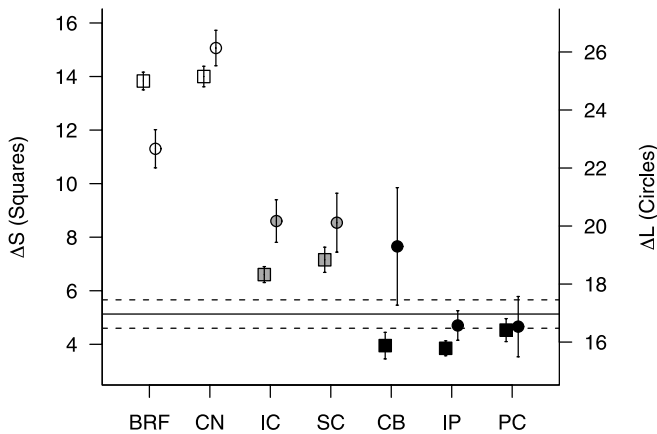
Figure 4. Weighted color contrast, ΔS, with standard errors indicated by squares scaled to the left axis. Horizontal lines indicate mean of all pairwise substrate color contrasts (solid) with standard error (dashed). Achromatic contrast, ΔL, with standard errors are indicated by circles and scaled to the right axis. Color contrast group is indicated with white, gray, and black symbols representing conspicuous, intermediate (conspicuous in the two group analysis), and cryptic, respectively.

Figure 5. Mean relative exposure (*Ac/As*) with standard errors for each locality. Symbols represent the three group division (triangles: conspicuous, squares: intermediate, circles: cryptic) and color represent the two group division (empty: conspicuous, filled: cryptic).

should however be treated with caution for two reasons. First, the reflectance of the individuals in CB was very low, which increases the uncertainty by measurement noise as indicated by the high variance in ΔL although low variance in ΔS. Especially, the calculations of ΔL are sensitive to this noise as the range of the double cone spectral sensitivity is less than that of the whole spectrum. Second, individuals in CB show contrast to the background, as they are darker and not brighter (as in the conspicuous populations) than the background. On the forest floor that is naturally speckled with dark holes and shadows it is much harder to locate darker objects than brighter ones. Thus, the contrast values obtained from a dark substrate and an even darker frog is potentially a much less-effective visual signal than that of an equally brighter frog.

Our choice of a quantum flux value of 10,000 is intended to be a fair estimate for a medium-sized violet-sensitive bird under the measured light levels at the forest floor. As the light intensity varies greatly in tropical forests, the chosen value of light intensity is somewhat arbitrary. However, sensitivity testing of this parameter in a range from 100 to 50,000 showed that only the magnitude but not relative visibility changed when varying intensity value. Because the analysis depends on relative visibility, the results of the study would thus not be affected by this confounding factor.

Our previous grouping of the populations into color contrast groups was therefore supported by the spectrum-based model. In this analysis of conspicuousness, we did not account for black patterns, but it is worth noting that the Isla Colon frog population that shows the lowest value for the aposematic group, and the Bastimentos frog population exhibit a low number of big and
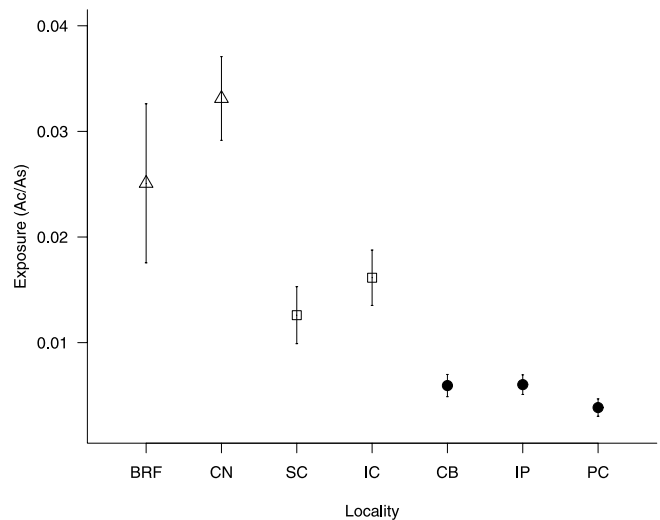
well-defined spots on their back which might enhance the signal of the bright color (e.g., Forsman and Merilaita 1999; Stevens et al. 2008). This grouping is congruent with the grouping into "Bright" and "Dull" of Wang and Shaffer (2008) except for Isla Colon (note that Solarte and Cayo Nancy are the same island). Two of the populations (IC and SC) that were classified as conspicuous showed ΔS values clearly lower than the other two (Fig. 4). Thus, we decided to include a second way of grouping the populations in the analysis of exposure. In this second approach, the intermediate populations (Isla Colon and Isla San Cristobal) formed a third intermediate group (Table 1).

EXPOSURE

We obtained exposure values based on 118 observations (Table 1). The individual relative exposure values ranged from 0 to 0.118 representing an approximate incident light level of 0% to 11.8% of the sun reference light. The population mean relative exposure values for all conspicuous populations (including intermediate) were higher than those for cryptic populations (mean of conspicuous population means: 0.0217, SE = 0.0046, $n = 4$, mean of cryptic population means: 0.0053, SE = 0.0007, $n = 3$, Fig. 5). The effect of color contrast group with locality as a random factor on male exposure was significant in both the two- and three-group approach (Table 2). The analysis of absolute exposure (*Ac*) confirms the results found for relative exposure, where the individuals in the conspicuous group were more exposed than individuals in the cryptic group (Fig. 6, Table 2). The difference was however no longer significant for our three-group approach. Exposure henceforth refers to neither of these specific definitions but rather to a more general meaning of visibility.
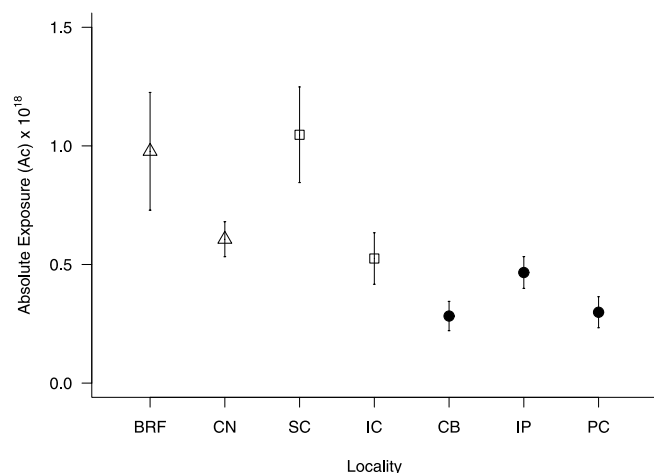
Figure 6. Mean absolute exposure (*Ac*) with standard errors for each locality. Symbols represent the three group division (triangles: conspicuous, squares: intermediate, circles: cryptic) and color represent the two group division (empty: conspicuous, filled: cryptic).

Table 3. ANOVA table of the results from phylogenetic generalized least squares (PGLS) analysis of relationships between conspicuousness group and relative exposure (*Ac/As*) and absolute exposure (*Ac*).

	Num df	Den dF	*F*-value	*P*-value
$(Ac/As)^{0.25}$				
Two groups	1	5	21.167	0.006
Three groups	2	4	46.219	0.002
$Ac^{0.25}$				
Two groups	1	5	1.500	0.275
Three groups	2	4	0.795	0.512

When treating the population on Pelican cay as closely related to Isla Pastores, all three cryptic populations represent independent losses of bright color (Wang and Shaffer 2008). Further, the PGLS analysis show similar results to that of the nonphylogenetic analysis, with significant effect of color contrast group on male relative exposure but not on absolute exposure (Table 3). A total of 11 random measurements of forest light were taken at five locations to control for general differences between conspicuous and cryptic locations. The individual exposure value of these measurements ranged from 0.011 to 0.085 and groups did not differ (cryptic: mean = 0.049, SE = 0.014, $n = 4$, conspicuous: mean = 0.037, SE = 0.009, $n = 7$, $t_{5.7} = 0.69$, $P = 0.52$).

Discussion

We measured the level of exposure during male sexual display in seven populations of *D. pumilio* assigned to different conspicuousness groups based on the mean population color contrast and achromatic contrast. Our results show that males belonging to populations with bright-colored individuals, display at more exposed sites than males from populations with more cryptic individuals. The visual analysis, weighted by observational data on the substrates used by frogs, and compared with natural substrate variation show that even though most color morphs in this species have at least one color component that contrasts enough against some substrates to be detected by a predator (Siddiqi et al. 2004) some of them fall within or even below the variation of the natural substrate. This means that they have a cryptic appearance that should make them very hard to detect visually. These results provide support for the view that not all populations are aposematic but that they represent two different predator avoidance strategies (Wang and Shaffer 2008). This conclusion has implications to earlier hypotheses on population divergence in this species (discussed in detail below).

Generally, the levels of exposure were low and one might argue that a mean of 2% relative exposure of males from the conspicuous group cannot be considered to reflect a high exposure. However using the random measures at localities we can roughly estimate incident light level in the species habitat to approximately 1–8% of that in open areas. With this in mind the difference in mean relative exposure between 2% for the conspicuous group and 0.5% for the cryptic group is more striking. To be more exposed than this, the individuals would have to move either higher in the canopy or out in grasslands or other open areas, both of which are unlikely due to physiological (e.g., thermal stress and desiccation) and ecological reasons (territorial properties e.g., spawning sites, food availability etc.).

An important assumption for our argument is that increased exposure is beneficial for male mating success. This is not only a key assumption in models of sexual selection (reviewed in Andersson 1994) but has also been found in this species. Pröhl and Hödl (1999) found a correlation between mating success and calling activity and average perch height. Moreover, Maan and Cummings (2009) have shown that male brightness, manipulated by different lightning regimes, affect female association behavior. Males in this species thus appear to gain mating success from both display behavior and coloration associated with higher exposure. It is also worth noting that males from populations with intermediate color contrast (Isla Colon and Isla San Cristobal) show intermediate degrees of exposure when calling. This could reflect that warning coloration and crypsis represent two extremes of a gradient of strategies (Mappes et al. 2005). Although, in this system and in other species where warning coloration not only provides an alternative predation avoidance strategy but also relieves male sexual display from constraint by predation, we predict that evolution of striking warning coloration may be rapid once this evolutionary path is taken and that populations with intermediate color contrast may be in a

transitional evolutionary stage. In other words, aposematic individuals could gain an immediate individual benefit through increased conspicuousness to potential mates, through their freedom to choose more conspicuous calling sites. This could offer a simple route to rapid evolution of aposematism.

Although it is intuitive that coevolution implies fast evolutionary gain of aposematic coloration—how will these two selective forces interact during the loss of aposematic coloration? This is a relevant question because several of the island populations of the strawberry poison frogs have apparently changed toward a lower degree of aposematic coloration (Wang and Shaffer 2008). There are many mechanisms that have been proposed to be responsible for phenotypic divergence in this system. First, although drift have been suggested to possibly act together with selection (Summers et al. 2003; Reynolds and Fitzpatrick 2007; Rudh et al. 2007; Maan and Cummings 2009) patterns of genetic, geographic, and morphologic distance between populations suggest that drift is unlikely to be the main reason for the striking phenotypic divergence and that neutral divergence alone is not sufficient to explain the existing variation (Rudh et al. 2007; Brown et al. 2010; Wang and Summers 2010).

Second, variation in natural selection including different ecological conditions (Maan and Cummings 2009; Brown et al. 2010) for example, predator communities (Reynolds and Fitzpatrick 2007, but see Summers et al. 1997), varying light conditions (see Summers et al. 2003 for arguments against) or fluctuations in toxicity (e.g., Daly and Myers 1967; Saporito et al. 2004, 2006, 2007a) may have lead to evolution of different color morphs. However, little is known about variation in ecological conditions of the areas inhabited by the different populations. The small geographical scale lowers the probability of an important role of variation in predator species distribution, especially if one considers the mobility of avian predators. Further, Summers et al. (1997) compared *D. pumilio* with two other species of poison frogs throughout Bocas del toro and found that these species showed a similar aposematic appearance throughout the range. This suggests that there has been a shift in morphological appearance even though predation is present. The ability to obtain toxins has been suggested to differ between environments and could be one possible catalyst for evolution of the remarkable color variation among populations of *D. pumilio* (e.g., Daly and Myers 1967; Saporito et al. 2004, 2006, 2007a). However, although frogs from all populations contain some toxin in their skin (which is a prerequisite for the evolution of aposematism) there is no clear correlation between the level of toxicity and coloration between these populations.

Third, sexual selection by female mate choice and its possible role in population divergence and/or maintenance of the population differences have been studied extensively (Summers et al. 1997, 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings

2008, 2009). This species (compared to two sympatric poison frog species) has both pronounced phenotypic divergence and greater female parental investment, suggesting stronger sexual selection (Summers et al. 1997). Females prefer to associate with individuals of their own color morph (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008) and have been found to prefer brighter males in some populations (Maan and Cummings 2009). Recent theory (Tazzyman and Iwasa 2010) suggests that runaway sexual selection could explain divergence in this system under specific conditions. Further, a suggestion for how directional sexual selection on brightness can affect hue has been proposed by Maan and Cummings (2009). We agree that this mechanism could explain some differences in hue within the conspicuous range of coloration. However, these hypotheses and models of sexual selection do not thoroughly address the shift in some populations from conspicuous to cryptic coloration. Female preference for brighter males would oppose evolution toward cryptic appearance, indicating the need to invoke changed natural selection. We propose that selection for crypsis alone may have lead to the variation in hue, as a result of achieving this by different genetic pathways. Anuran coloration is formed by a combination of pigments and structures (Bagnara et al. 1968; Frost and Robinson 1984) and is controlled by several loci (Summers et al. 2004) and possibly several control regions. Thus, a loss of bright red coloration could likely be achieved in many different ways, resulting in several different hues (see Summers et al. 2004 for some discussion on proximal causes of coloration).

Fourth, because the adaptive value of aposematic coloration depends on the strength of the learned aversion of the predators (reviewed in Ruxton et al. 2006) the benefit of being aposematically colored (for each individual) is predicted to be reduced when the population size is small. Therefore, a change in population size may lead to a shift in selection on the predation avoidance strategy (i.e., dorsal coloration) even if all other factors are kept constant. In the first three cases outlined above, sexual selection would probably slow down the loss of aposematic coloration but possibly still facilitate population divergence through a role in sexual isolation (see below). However, given the fourth scenario, a weakening of sexual selection could reinforce the effect of the directional change in natural selection. The Kaneshiro model (1989) on peripatric speciation suggests that sexual selection should be relaxed following population bottlenecks at the leading edge of an expansion or during the colonization of islands. This is because low population size may lead to reduced variation in mating success that is, relaxed sexual selection (Kaneshiro 1989; Ardren and Kapuscinski 2003). There are several possible biological reasons for why a relaxation in sexual selection should happen under low population densities and colonization as described above, and one relevant suggestion is that the need to defend breeding territories is reduced when the breeding habitat is unsaturated with

individuals (Ödeen and Björklund 2003). This is relevant in *D. pumilio* as both sexes are territorial. Females choose their territories in accordance with the presence of tadpole rearing sites (small water bodies in leaf axils), and males in accordance with the presence of females (Pröhl and Berke 2001). Males with such territories are highly aggressive toward conspecific males (Pröhl 2005). Thus, a decreased populations size could lead to both relaxed sexual selection for conspicuous males, and to a shift in natural selection to benefit dull rather than aposematic individuals, joining both types of selection to act toward a loss of aposematism (despite the level of toxicity). In conclusion, we consider changes in selection pressures associated with the fragmented habitat in Bocas del Toro (caused by changes in density of frogs and possibly also in the predator fauna and availability of toxin) to be a likely explanation for the initial switches in the direction of the coevolution of coloration and sexual display in the different populations of frogs. However, because these selection pressures are likely to change over time, it is impossible to make any firm conclusions about the underlying reasons for the initial shifts in direction of evolution of these traits.

Sexual selection is assumed to act as a strong force driving speciation because population divergence in sexually selected traits can lead to sexual isolation between them (West-Eberhard 1983; Price 1998; Panhuis et al. 2001; Ritchie 2007). One can argue that population divergence in male sexual display need not necessarily lead to sexual isolation if there is not a corresponding change in female preference. However, there are several reasons to assume that differences in male sexual display in *D. pumilio* increases their likelihood of pairing with females using the same predator avoidance strategy. For example, a loss of conspicuousness should restrict a female's willingness to expose herself while visually, accompanied by calls, locating and tracking males to proposed spawning sites (Limerick 1980). Further, the female has to move around, both to find a rain-filled leaf axis or other small body of water where tadpoles are raised, and while feeding tadpoles with unfertilized eggs (Weygoldt 1980; Brust 1993). In Panama populations of *D. pumilio*, have become isolated very recent and at least some populations produce viable offspring (Summers et al. 2004). However, females already tend to associate with individuals of their own color morph, based on visual cues, indicating a rise of reproductive isolation between populations (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008). Further studies are needed to evaluate the present levels of both prezygotic and postzygotic reproductive isolation. We show that divergence in coloration correlates with a difference in behavior, which could in turn affect mating behaviors and maternal care for the offspring. For example, if both males and females are conspicuous, and males are driven by competition to display in exposed sites, females will have to visit the same area as the males to mate and to deposit their eggs. If a female is

cryptic she would be likely suffer from increased predation if she is sharing "mating space" with a conspicuous male. Thus, sexual isolation may result from a matched degree of exposure during mating of males and females, which in turn could cause sexual selection against migrants with a deviating strategy. The crucial question then becomes how important this phenomenon would be in other speciation events. Phylogenetic investigations of evolution of aposematism are sparse and aimed at variable species groups. Multiple evolution of aposematism has been found in poison frogs (Hagman and Forsman 2003; Santos et al. 2003; Vences et al. 2003) and Malagasy frogs (Schaefer et al. 2002). Loss of aposematism in speciose groups are probably more common as this does not require all the conditions that a gain of aposematism does, although we do not know of any study that systematically has investigated how common it is. Nevertheless, the importance could potentially be big even if shifts between the two strategies are likely to be slower than in the strawberry poison frogs (see Przeczek et al. 2008) for some examples of taxa with both aposematic and cryptic species). Moreover, the same situation would apply for closely related species that differ in their antipredator strategy, including Batesian mimicry (e.g., Kuchta et al. 2008).

In general, questions related to how natural and sexual selection interact at different stages of the speciation process need further empirical attention. According to theory, naturally selected traits that also affect behaviors involved in sexual display and mating should favor the evolution of complete reproductive isolation. Particular types of naturally selected traits may be more likely to coevolve with sexually selected traits or may even cause sexual isolation directly as a side effect (Via 1999) or through a dual function of the trait (Nagel and Schluter 1998; Eizaguirre et al. 2009). A recent shift in mimicry color pattern between the butterfly sister species *Heliconius melpomene* and *H. cydno* has been shown to cause reproductive isolation by assortative mating (Jiggins et al. 2001). Similar to this, color in *D. pumilio* tends to act as a "magic trait" (Gavrilets 2004) that directly causes assortative mating by mate choice (Reynolds and Fitzpatrick 2007; Maan and Cummings 2008). Our results suggest that color also affects sexual display behavior. Because the change in the ecological trait (aposematic vs. cryptic coloration) in this case by itself is assumed to cause a change in natural selection on the display behavior (i.e., the predation cost associated with exposure), the build up of the association between these traits would not be sensitive to gene flow and may function as a starting point for the evolution of reproductive isolation between color morphs.

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