ORIGINAL PAPER

Conspicuous displays in cryptic males of a polytypic poison-dart frog

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Abstract The evolution of aposematism is linked to increased opportunities for conspicuous sexual displays since detection by potential predators is no longer disadvantageous. Therefore, phenotypic divergence in aposematic species leading to relatively cryptic forms is expected to constrain such opportunities, by restoring the trade-off between natural and sexual selection on the boldness of sexual displays. We asked if and how a derived phenotype of the poison-dart frog *Oophaga granulifera* that appears relatively cryptic to potential predators exhibits conspicuous sexual displays for potential mates. We used visual modeling of frog contrasts against their natural backgrounds to test if for conspecifics green frogs appear less conspicuous than red frogs as they do for birds. We conducted behavioral observations of focal red and green males to determine if green frogs adjust their display behavior to the availability of potential mates. Dorsal brightness is known to influence female preferences in at least one poison frog species. We found that, despite being less visible under some measures, green frogs may appear as bright as red frogs for conspecifics but not birds, when viewed on dark backgrounds. Additionally, green males called more intermittently than red males when advertising to distant females, but they exhibited a dramatic increase in calling activity in proximity of a female and were as active as red males in this context.

Communicated by M. Gibbons

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Together, our results suggest that green frogs retain context-dependent conspicuousness to conspecifics despite the evolution of relative crypsis to potential predators.

Introduction

Theory contends that the expression of conspicuous sexual displays will be favored by sexual selection but limited by natural selection in most prey (Andersson 1994) because potential mates tend to select for increased sensory stimulation (Ryan and Keddy-Hector 1992; Dawkins and Guilford 1996) while predators will more likely detect and attack animals engaged in conspicuous displays (Magnhagen 1991; Zuk and Kolluru 1998; Godin and McDonough 2003). Therefore, the evolution of aposematism offers a possibility of relaxing this trade-off as conspicuous displays should no longer entail high risk of predation. Indeed, the widespread evolution of warning signals in noxious prey appears to be enhanced by the non-predation benefits of living a bold life (Merilaita and Tullberg 2005; Speed and Ruxton 2005; Speed et al. 2009). Nonetheless, the evolution of cryptic phenotypes from aposematic ancestors occurs in nature. How these novel morphs cope with the predicted reduction of opportunities prompted by crypsis is unknown.

The evolution of crypsis from aposematic ancestors may incur opportunity costs for mate signaling, if it also implies reduced conspicuousness to conspecifics during sexual displays. Furthermore, because the effectiveness of visual crypsis depends on background matching and behavior (Ioannou and Krause 2009), behavioral and morphological components of sexual displays may be constrained by predators in cryptic

animals. However, non-aposematic species are known to use numerous solutions to deliver conspicuous signals to potential mates while remaining relatively cryptic to potential predators (Magnhagen 1991; Endler 1992). For example, prey may engage in conspicuous courtship displays only in times and places where visual conspicuousness to predators is low (e.g., Endler 1987; Kotiaho et al. 1998; Jones et al. 2002). Prey may also reduce detection risk if they signal to mates intermittently (Endler 1992), or adjust the frequency or intensity of a display according to current mating probability (Wing 1988; Kålås et al. 1995; Candolin 1998). Further, signals may be transmitted through private channels of communications, using sensory modalities imperceptible for predators (Cummings et al. 2003), or through the same channels but tuned to receiver-specific sensitivities (Endler 1991; Håstad et al. 2005). Here, we asked if male poison-dart frogs of a derived relatively cryptic phenotype retain conspicuousness to females by (1) using visual signals tuned to sensitivity of conspecifics but not predators and/or (2) adjusting behavioral display conspicuousness to the availability of potential mates.

Neotropical poison-dart frogs have become excellent model organisms to study the ecological significance of phenotypic divergence. In at least two species of the genus *Oophaga*, color divergence spans a large spectrum of conspicuousness to potential predators that is correlated to behavioral conspicuousness (Pröhl and Ostrowski 2011; Rudh et al. 2011; Willink et al. 2013) and may be directly (Maan and Cummings 2012) or inversely (Wang 2011) correlated with toxicity. *Oophaga* females bear a major investment in parental care by providing unfertilized eggs as food to developing tadpoles (Summers et al. 1999a), hence are expected to exert strong selection on male traits (Trivers 1972). Calling activity is thought to influence mating success of males of several species of poison frogs (e.g. Roithmair 1994; Pröhl 2003). Also, visual signals are important in poison frog communication, during male–male interactions (Narins et al. 2003; Crothers et al. 2011) and courtship (Silverstone 1973; Montanarin et al. 2011), and laboratory studies show that females attend to dorsal coloration in their mating decisions (Summers et al. 1999B; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008, 2009; Richards-Zawacki and Cummings 2011). Thus, it seems reasonable that the behavioral and coloration components of display conspicuousness are simultaneously subject to selection by predators and mates.

We studied male sexual displays in two populations with divergent anti-predator strategies of the granular poison-dart frog *Oophaga granulifera*. The ancestral red phenotype is highly conspicuous to the vision of potential bird predators compared to a derived green morph (Wang 2011; Willink et al. 2013). To test the hypothesis (1) that visual conspicuousness of green frogs is tuned to the sensory system of frogs we assessed how visual contrasts between both frog phenotypes and their backgrounds are perceived by conspecifics as well as

potential predators. If crypsis to predators does not require decreased visual conspicuousness to potential mates, there would be no trade-off between natural and sexual selection in this signaling modality. To test the hypothesis (2) that green males adjust their behavioral conspicuousness to mate availability, we compared the calling activity and position of red and green males in an advertisement context (to attract potential distant mates) and a courtship context (when mate availability is certain). We expected green males to match the behavioral conspicuousness of red males only in the courtship context.

Methods

Study species

The granular poison frog, *O. granulifera*, is distributed along the southwestern lowlands of Costa Rica (Savage 2002), where yellow and green color morphs have evolved from red ancestors (Wang 2011; Brusa et al. 2013). We studied one population in the northern end of the species distribution (9°35' N, 84°13' W) where frogs exhibit dark and dull green dorsal coloration, and one population 37 km southeast (9°16' N, 83°52' W) where the dorsal coloration of frogs is bright red (Willink et al. 2013; Fig. 1). The ventral coloration is turquoise at both localities, but brighter in the dorsally red frogs (Willink et al. 2013). Males of *O. granulifera* call throughout the rainy season (May–November) although activity peaks during the first weeks of rain (Bolaños 1990). Most calling activity takes place early in the morning (5:30–9:00) and again in the late afternoon (16:30–18:00), but mating normally occurs in the morning (BW, personal observation). Males can be repeatedly found in territories that are defended by vocalizations and occasionally physical combat (Goodman 1971; Crump 1972). Moreover, frogs exhibit remarkable variation in the distribution of turquoise areas on their legs and ventral surfaces, allowing individual recognition with photographs.

Coloration conspicuousness

We used visual modeling developed by Vorobyev et al. (1998) and modified by Siddiqi et al. (2004) to assess the conspicuousness of calling males to conspecific frogs and a model predatory bird. Birds are considered potentially important frog predators as they readily attack clay models of *O. granulifera* in the field (BW et al., unpublished data) and are known to prey on other poison frogs (Master 1999; Alvarado et al. 2013). As measures of visual conspicuousness, we modeled the chromatic (ΔS) and achromatic (ΔL) contrasts between the skin of frogs and their natural backgrounds, and calculated the overall conspicuousness as the Euclidean distance from the origin of perceptual space and (ΔS) and (ΔL) as Cartesian coordinates. The inputs of the visual models were the reflectance spectra of

Fig. 1 Displaying males of a red and a green population of *O. granulifera*

frogs and their natural backgrounds, the irradiance spectra at frog calling sites and the sensitivity spectra of frog and bird receptors (Fig. 2). Contrasts were calculated over 300–700 nm, with an integration interval of 1 nm. To model chromatic contrasts, we assumed that thresholds for color discrimination depend only on the relative noise of cone channels (i.e., Weber law holds), where photoreceptor noise (ω) is a function of the noise-to-signal ratio for each cone class (ν) and the relative number of cone types in the retina (Vorobyev and Osorio 1998). To model achromatic contrasts, we assumed that luminance discrimination is performed by long-wave-sensitive (LWS) cones in frogs and double cones, which contain LWS pigments, in birds (Siddiqi et al. 2004).

Reflectance spectra were obtained using an Ocean Optics bifurcal optic fiber R-200-2-UV/VIS connected to an Ocean Optics HR2000 spectrometer and a deuterium-tungsten DT-Mini-2-GS as a light source. A WS-1-SS white standard was used for calibration every eight measurements to account for lamp drift. We measured dorsal and ventral reflectance of 22 focal males and of all backgrounds they used when calling (see below). Reflectance spectra were averaged over eight measurements per frog surface and five measures per background ($n = 28$ backgrounds used by green frogs and 34 by red frogs). Background substrates were grouped into six categories: (1) trunks, including all types of tree bark, (2) rocks, normally covered with moss, (3) leaf litter, (4) green leaves, (5) banana stems, and (6) bare soil. Banana plants have similar reflectance to trunks (Online Resource 1), but they were only available in the red locality. Bare soil, although readily available, was never used as a calling background by green males. We present the mean visual contrasts of males and the four backgrounds used by both populations; however, our results are unchanged by modeling frog conspicuousness on bare soil (Online Resource 2). Irradiance spectra were obtained by attaching an Ocean Optics cosine adaptor head (CC-3UV) to an optic fiber QP-400-2-UV-BX connected to the HR2000 spectrometer. Light irradiance was measured twice during the hours of frog activity at 6:00 and 9:00, but both measures were averaged to calculate contrasts since they generated the same

qualitative results. To calculate the visual contrasts of frogs, we used the mean irradiance spectra from each population ($n = 25$ calling sites at each population).

The sensitivity spectra of the closely related *Oophaga pumilio* were used to model frog vision because spectra for *O. granulifera* cones are not available. We considered the sensitivity spectra of the bird *Parus caeruleus* as appropriate since only a few species of birds have been recognized as potential predators of *O. granulifera* (BW et al., unpublished data), and in 26 species investigated, spectral sensitivities of birds did not show much variation (Hart 2001). Birds have either UV-sensitive, as *P. caeruleus*, or violet-sensitive vision. Here, we use UV-sensitive vision only since conspicuousness estimates of poison frog coloration are similar for both systems (Maan and Cummings 2012).

Behavioral conspicuousness

A single observer conducted behavioral observations of 12 red and 10 green focal males. Each male was observed for 15 min on ten different days (i.e., ten observations per male) between 05 September and 12 November 2011. Observations were conducted in a randomized order at each population between 5:30 and 10:00. Normally, six to eight observations were completed in one morning, and we only conducted observations when the focal male could be seen from a distance of at least 2 m and it did not hop away from the approaching observer prior to data collection. For each individual, we recorded the time (seconds) that the frog spent calling (calling activity), the substrate(s) where the male had called from, and whether or not courtship was occurring during the observation. For a particular male, courtship occurred if he called while directly facing a nearby female and/or followed her as to get closer and present himself in front of the female. In our observations, this happened when the female was at an initial distance of less than 2 m from the focal male. After an observation had finished, we measured the height and the exposure of the calling positions used by the male and collected samples of the substrates at these positions to obtain

Fig. 2 Data used to model visual conspicuousness of frogs, for bird and frog viewers: mean dorsal (**a**) and ventral (**b**) reflectance of the skin of red and green focal males, mean reflectance spectra of the most common backgrounds at the green (**c**) and red (**d**) populations, mean irradiance spectra at the green (**e**) and red (**f**) populations, and estimated sensitivity spectra of the receptors of *O. pumilio* (**g**), courtesy of T. Cronin, and the model predatory bird *P. caeruleus* (**h**), courtesy of N. Hart. *Shaded areas* show the standard error of means
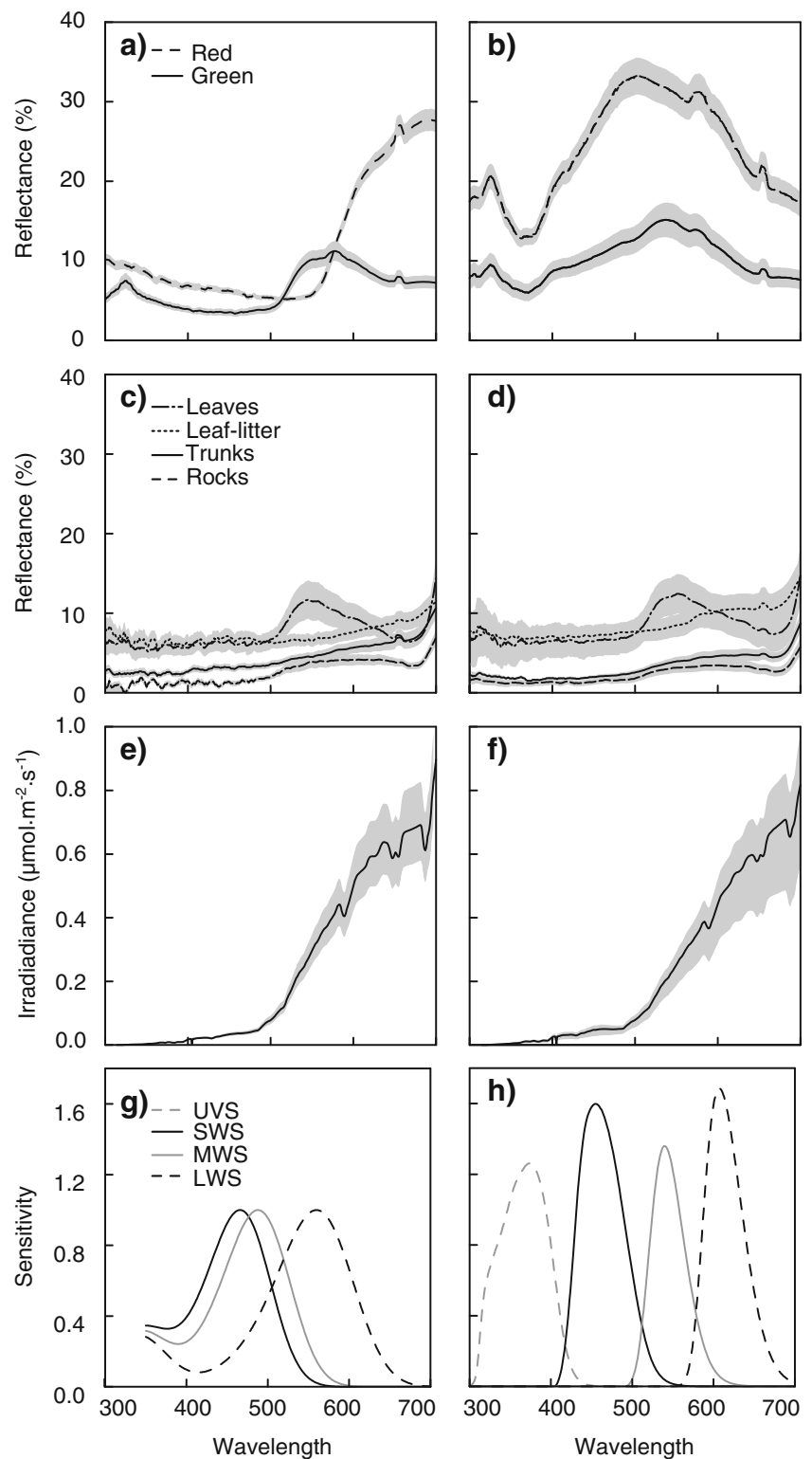
reflectance spectra. To measure exposure, we placed a frog clay model in the exact positions the male had called from and took five pictures at distances of 1 m from the model. Four of the pictures were taken placing the camera at the same height as the frog model (one from the front, the back, and each side), and one picture was taken from right above the model. We downloaded the pictures and estimated the proportion of the frog model (0, 0.25, 0.5, 0.75, or 1) that was visible in the pictures compared to pictures taken in a completely open area. The five measures of exposure were averaged for each

position, and a final exposure measure was calculated for each male as the mean exposure at all calling positions weighed by the time spent at each position.

Statistical analyses

All analyses were conducted in R (R Development Core Team 2009). We used linear mixed-effect models (LMMs) to evaluate the visual conspicuousness of frogs. As fixed factors, we use the frog phenotypes (green and red), the backgrounds (trunks, rocks, green leaves, and leaf litter), and the viewer classes (frog and bird). Because the same frogs were used to model conspicuousness for different viewers, we used the individual frogs as a random factor, nested within the populations. We conducted separate analyses for the chromatic and achromatic contrasts of the dorsal and ventral skin of the frogs. We report uncorrected P values and P values adjusted for the false discovery rate (FDR) to control for alpha-inflation under multiple tests (Benjamini and Hochberg 1995). If frogs have visual signals tuned to conspecifics but not to predators and high conspicuousness to females is favored in both populations, we would expect a large difference in conspicuousness between morphs for bird viewers but not for conspecifics. Alternatively, if assortative mating has contributed to color divergence in this species and differences in conspicuousness are informative for female choice, then larger differences in conspicuousness could be expected for conspecifics than for potential predators. A significant interaction between the frog phenotype and the viewer class would indicate that differences in conspicuousness between morphs are viewer dependent.

To determine if green males adjust the conspicuousness of their displays to the presence of mates in close proximity, we tested the effects of frog phenotype and social context (i.e., courtship if a female was available, advertisement otherwise) on the behavioral elements of male displays. For each male, we averaged the calling time, exposure, and height of position across all observations of each context. The mean values for each male were used in two-way ANOVAs testing the effects of frog phenotype, social context, and their interaction. Calling activity data was log transformed, and visual exposure was arcsin of square root transformed to meet the statistical assumptions of the analyses.

Results

Coloration conspicuousness

Dorsally, red frogs are overall more conspicuous than green frogs, but the magnitude of this difference depends on the viewer class (Table 1). Birds perceive larger differences in frog overall conspicuousness between the red and green phenotypes, while for conspecifics red and green males are similarly

conspicuous on most backgrounds (Fig. 3). Taken separately, the two components of overall conspicuousness reveal different patterns. In terms of chromatic contrast, the difference in conspicuousness between color morphs is more pronounced for frog vision. Regarding brightness contrast, green frogs are relatively more conspicuous to conspecifics than to potential predators, while only for bird vision red frogs are consistently more conspicuous than green frogs (Fig. 4). Indeed, for the eyes of frogs, red and green males are similarly bright when seen on trunks (mean difference=2.31, $CI_{95\%}$=[−4.23–8.85]) and rocks (mean difference=2.56, $CI_{95\%}$=[−3.99–9.10]), the most commonly used backgrounds (Online Resource 1). In both types of contrasts, dorsal conspicuousness also depends on the background substrate, and apparent brightness further depends on the interaction between the background and phenotype (Table 2): green frogs appear relatively brighter on dark backgrounds (i.e., trunks and rocks; Fig. 4b,d) compared to other backgrounds.

Ventrally, frogs are more conspicuous to conspecifics than to birds, and there are no significant interactions between viewer class and frog phenotype (Tables 1 and 3). For the vision of both birds and frogs, red and green frogs have similar chromatic contrasts (Fig. 5), but red frogs appear brighter and have higher overall conspicuousness (Figs. 3 and 5). Ventral visual conspicuousness of frogs depends on the background substrate, but the patterns described above were consistent across substrates (Tables 1 and 3).

Taken together, our results show that the green frogs are as conspicuous as red frogs concerning overall dorsal conspicuousness and dorsal brightness contrasts on most used calling places. However, they are less conspicuous for both viewers in terms of dorsal color contrast, ventral brightness contrast, and ventral overall conspicuousness. In addition, the ventral color contrast was similar for both morphs and higher for frog than for bird vision.

Behavioral conspicuousness

Courtship occurred in 16 out of the 220 observations conducted. Nine courtship observations were on six red males, and the other seven were on six green males. Within each population, calling behavior, exposure, and perch height during advertisement did not differ between males observed only in this context and males observed in both contexts (paired t tests, all $P>0.22$). Thus, observations of all 22 males were used to estimate the behavioral components of advertisement displays in each population. Calling activity depended upon the frog phenotype ($F_{1,30}$=7.12, P=0.012), the context of the display ($F_{1,30}$=141.83, $P<0.001$), and their interaction ($F_{1,30}$=13.42, $P<0.001$). Both male phenotypes dramatically increased their calling activity with the presence of a female, from 5.7 % ($CI_{95\%}$=[4.2–7.6]) to 74.5 % ($CI_{95\%}$=[50.9–100.0]) in green males, and from 14.3 % ($CI_{95\%}$=[10.9–18.8]) to 56.6 %

Table 1 Results of LMMs testing the effects of frog phenotype, background, and viewer class on the dorsal and ventral overall conspicuousness of frogs on their natural backgrounds

	df	Dorsal conspicuousness			Ventral conspicuousness		
		F	P	Adjusted P	F	P	Adjusted P
Phenotype	1	49.11	**<0.001**	**<0.001**	101.00	**<0.001**	**<0.001**
Viewer	1	7.78	**0.006**	**0.012**	6.09	**0.015**	**0.003**
Background	3	28.10	**<0.001**	**<0.001**	23.27	**<0.001**	**<0.001**
Phenotype*Viewer	1	13.44	**<0.001**	**<0.001**	0.62	0.432	0.432
Phenotype*Background	3	0.85	0.467	0.902	0.19	0.902	0.902
Viewer*Background	3	0.19	0.905	0.905	1.17	0.323	0.646
Phenotype*Viewer*Background	3	0.69	0.559	0.666	0.52	0.666	0.666

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

(CI$_{95\%}$=[38.6–82.8]) in red males. However, the significant interaction between the male phenotype and context points to a greater increase of calling activity in the green males (Fig. 6a). Frog exposure while calling differed between phenotypes ($F_{1,30}=14.18$, $P<0.001$), but not between contexts ($F_{1,30}=3.17$, $P=0.085$), and there was no interaction between phenotype and context ($F_{1,30}=0.46$, $P=0.504$). On average, 59.1 % (95 % CI=53.0–65.2) of the body of displaying red males was visible from a distance of 1 m, while green males only exposed 42.4 % (95 % CI=35.9–48.9) of their bodies while calling (Fig. 6b). Males called from lower positions in the presence of a female ($F_{1,30}=25.6$, $P<0.001$, Fig. 6c), but perch height was similar for both phenotypes ($F_{1,30}=1.48$, $P=0.233$), and there was no significant interaction ($F_{1,30}=1.30$, $P=0.263$). Nonetheless, there was a statistical trend that advertising green frogs had higher calling positions than red males in this context (simultaneous tests, function glht in library multcomp $t=1.83$, $P=0.077$; Fig. 6c).

Discussion

Coloration conspicuousness

In non-aposematic animals, signal design results from a trade-off between signal efficiency to potential mates and predators (Endler 2000); hence, green frogs would benefit from reduced conspicuousness to predators but enhanced conspicuousness to conspecifics. Notably, this prediction would only hold if females select for greater signal quantity, and therefore, more conspicuous signals directed to conspecifics are advantageous in both populations. We found support for this hypothesis in the overall dorsal conspicuousness and dorsal brightness contrast of male frogs. Relative to red males, the green males of *O. granulifera* are more conspicuous to conspecifics than to birds, and on the two most common calling backgrounds, red and green frogs appear similarly bright to conspecifics. Conversely, green males have lower dorsal color contrasts than red males for both viewers, but especially for frogs.

Thus, these results may seem at odds with each other, suggesting both sensory tuning and constraints in a female's perception of conspecific green males. However, they could be explained without dismissing our hypothesis if different forms of sexual selection act on the two signaling traits, that is, if females favor greater signal quantity in dorsal brightness and mate assortatively based on dorsal hue.

Different lines of evidence suggest that male dorsal brightness is a target of selection for greater signal quantity in the closely related *O. pumilio*. First, *O. pumilio* females prefer males that appear brighter in laboratory trials (Maan and Cummings 2009). Second, it is possible that brightness acts as a signal of male quality, since it is correlated with body temperature and male displaying behavior (Crothers et al. 2011). Finally, within a population, variation in dorsal brightness is more detectable for conspecifics than for potential bird predators, suggesting that predator sensory constraints allows sexual selection to shape the evolution of this signal (Crothers and Cummings 2013).

On the other hand, female preferences for higher chromatic contrast have not been reported in *Oophaga*. Instead, it appears that females prefer to mate assortatively based on dorsal hue (Reynolds and Fitzpatrick 2007; Maan and Cummings 2008), and this preference may not be compatible with a preference for high chromatic conspicuousness. Indeed, green females of one population of *O. pumilio* prefer males of their own dorsal color rather than red males (Summers et al. 1999b) which have higher chromatic contrasts (Maan and Cummings 2012). Thus, if assortative mating has also influenced the evolution of color divergence in *O. granulifera* and differences in male chromatic conspicuousness are informative to females (or at least correlated with hue), large differences in conspicuousness between red and green males are expected for frog vision under the hypothesis of visual signals being tuned to conspecifics. Our results suggest that in *O. granulifera*, reduced conspicuousness to potential predators (i.e., a cryptic anti-predator strategy) has evolved without an equivalent loss in conspicuousness of a signal likely selected for greater quantity, the dorsal brightness contrast.

Fig. 3 Dorsal and ventral overall conspicuousness of frogs on their calling backgrounds: trunks (**a, b**), mossy rocks (**c, d**), green leaves (**e, f**), and leaf litter (**g, h**), as seen by conspecifics and potential bird predators. *Error bars* show the SE of means
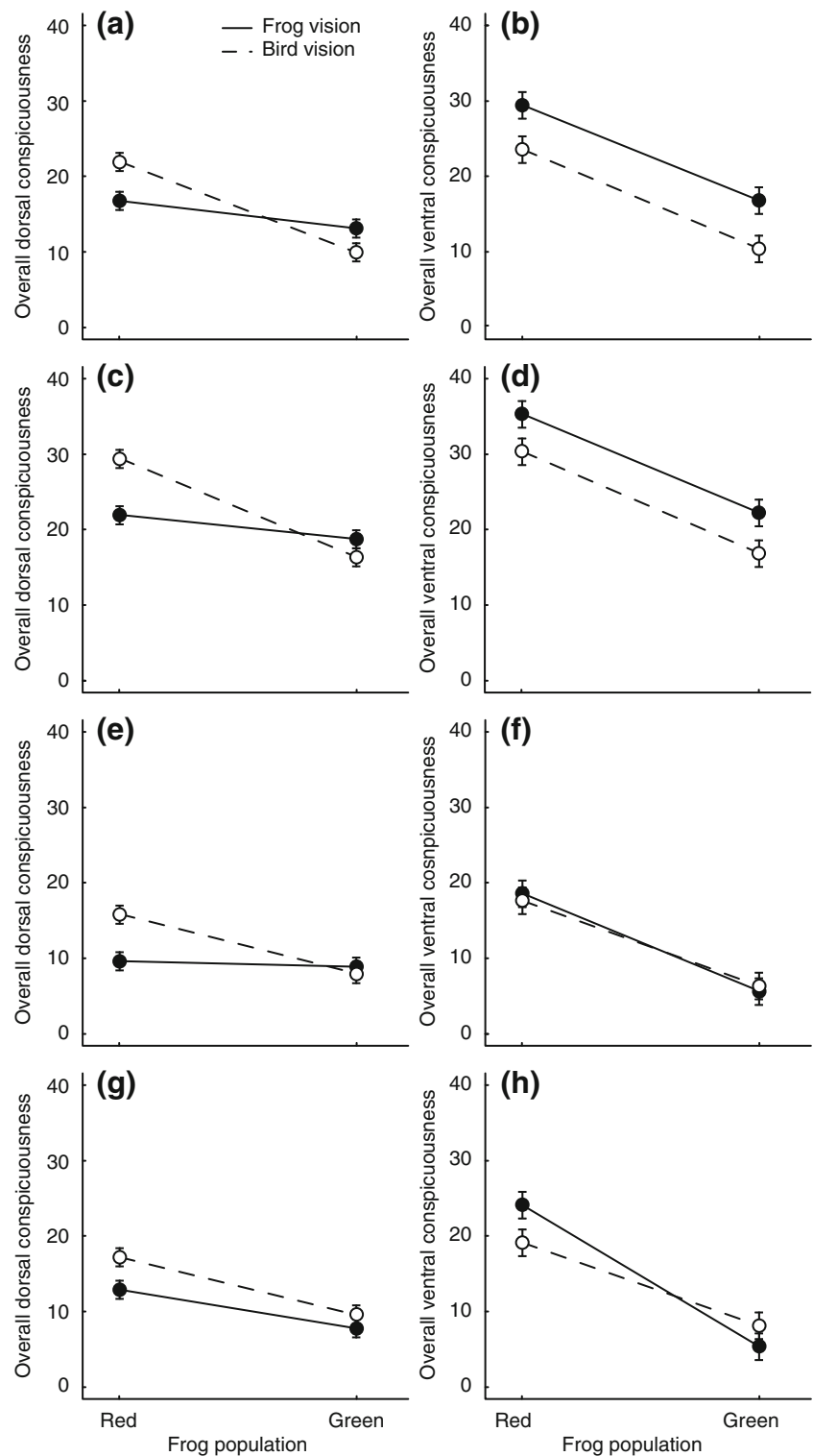
Ventrally, green and red frogs are similarly conspicuous in terms of color contrast, but green males are less conspicuous for both viewers in terms of brightness contrast and overall conspicuousness. Our finding that green frogs appear relatively bright to conspecifics dorsally but not ventrally differs from the results of a similar study with *O. pumilio*, where males of a red phenotype seem dorsally brighter to conspecifics, but green frogs of one population display high ventral brightness contrasts (Pröhl and Ostrowski 2011). The role of ventral signals in intraspecific communication of poison frogs has received less attention, but they may also be used as calling frogs adopt an upright position (Crump 1972). It remains

Fig. 4 Chromatic and achromatic visual contrasts between the dorsal skin of frogs and their calling backgrounds, as seen by conspecifics and potential bird predators. Contrasts were calculated on common calling backgrounds: trunks (**a, b**), mossy rocks (**c, d**), green leaves (**e, f**), and leaf litter (**g, h**). *Error bars* show the SE of means
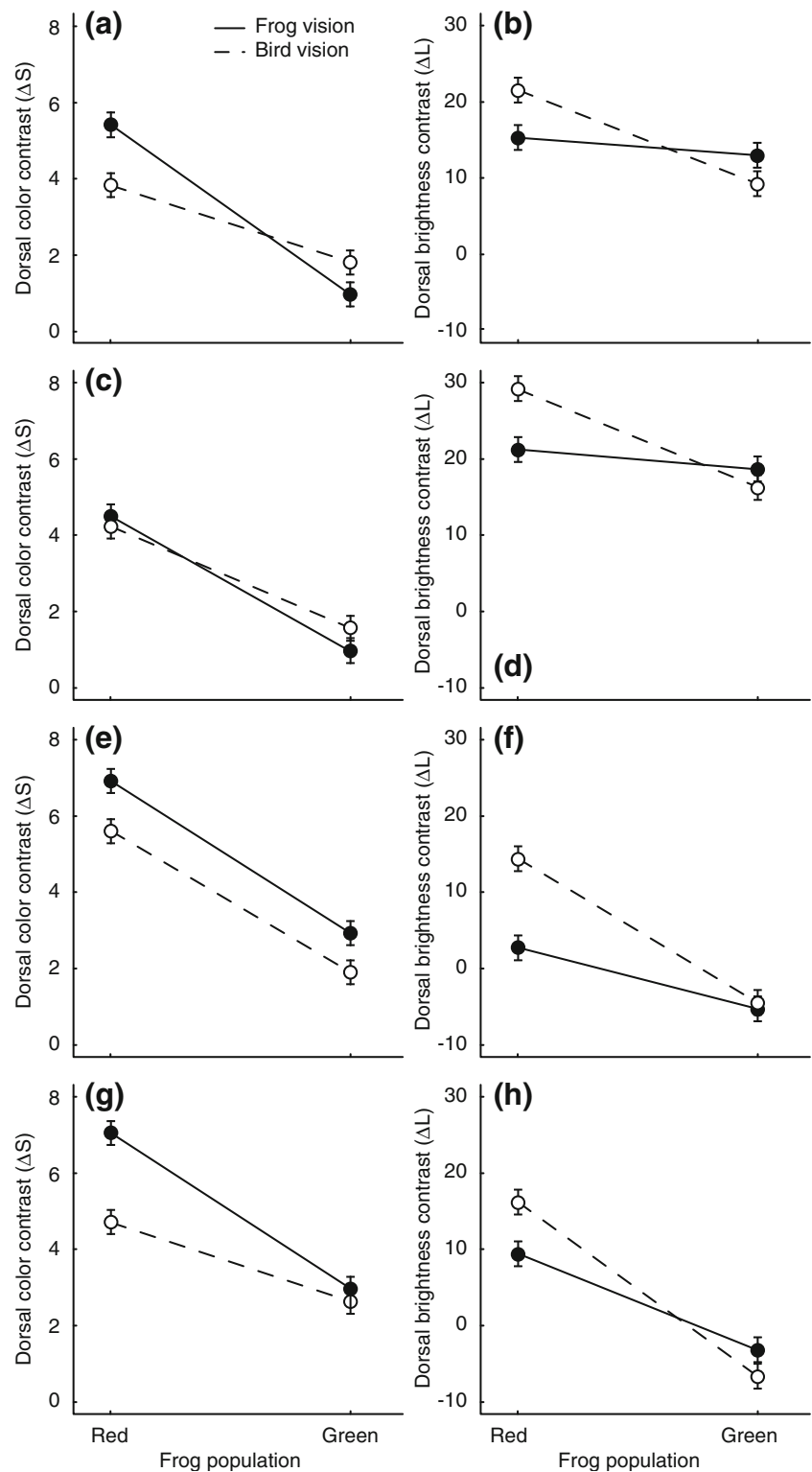
unexplored whether female preferences for bright signals involve the dorsal coloration only, as it seems to be for female preferences on male hue (Maan and Cummings 2008), or any bright signal.

Behavioral conspicuousness

Observations of male calling activity uncovered remarkable behavioral plasticity associated with social context. Most of

Table 2 Results of LMMs testing the effects of frog phenotype, background, and viewer class on the dorsal color and brightness contrast between frogs and their natural backgrounds

	df	Dorsal color			Dorsal brightness		
		F	P	Adjusted P	F	P	Adjusted P
Phenotype	1	193.56	**<0.001**	**<0.001**	102.17	**<0.001**	**<0.001**
Viewer	1	**9.76**	**0.014**	**0.003**	**9.21**	**0.003**	**0.003**
Background	3	12.44	**<0.001**	**<0.001**	62.69	**<0.001**	**<0.001**
Phenotype*Viewer	1	8.60	**0.004**	**0.008**	20.26	**<0.001**	**<0.001**
Phenotype*Background	3	0.58	0.628	0.628	4.80	*0.003*	*0.013*
Viewer*Background	3	2.23	0.087	0.240	1.02	0.386	0.514
Phenotype*Viewer*Background	3	1.07	0.362	0.999	0.008	0.999	0.999

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

the time, males are not interacting with potential mates, and advertisement calls are intermittently produced to defend the territory and attract distant females (Goodman 1971; B. Willink pers. obs.). Upon the arrival of a female, a mating opportunity becomes manifest, and males from both phenotypes increase their calling activity. Calling is energetically expensive for anurans that produce relatively loud calls for their size (reviewed in Wells 2007). Therefore, it is advantageous for males to only maximize the intensity of their displays when a mating opportunity (or a conflict with an intruder) appears immediate (Byrne 2008).

Advertisement calling activity of green frogs may be further restricted by predation risk. Green but not red frogs are expected to increase their own risk of predation by performing bold displays. This is because bold behaviors increase the rate of predator encounters (Kotiaho et al. 1998), while the strength of learned aversion of toxic prey (Gittleman and Harvey 1980; Roper 1994) and the ability to discriminate prey noxiousness (Guilford 1986; Darst et al. 2006) are associated with the conspicuousness of prey coloration. Accordingly, green males exhibited lower calling activity than red males in the absence of a female. Green males also have reduced

feeding frequency, probably because active foraging increases conspicuousness to potential predators (Willink et al. 2013). Consequently, even if both frog phenotypes would benefit from increasing the intensity of sexual displays when a female is nearby, increased calling activity should be more costly for green males, both energetically, since they have lower foraging rates, and in terms of predation risk. However, our results show that despite this additional cost, green frogs exhibit a steeper increase in calling activity during courtship (13-fold compared to 4-fold in red males), and they spent as much time calling as red males did in this context. This is particularly relevant because in poison-dart frogs with strong sexual selection calling rate during courtship is an honest predictor of male genetic quality (Forsman and Hagman 2006).

Males changed their position but not their degree of exposure to engage in courtship. Females approach a territory hopping on the leaf litter, and if courtship is successful, mating will occur inside a bowed leaf of the leaf litter (Crump 1972; BW and FB personal observation). Thus, males move to lower positions to engage in courtship, and green males did so while remaining more concealed than red males by vegetation and other habitat structure. It is not entirely clear from our data

Table 3 Results of LMMs testing the effects of frog phenotype, background, and viewer class on the ventral color and brightness contrast between frogs and their natural backgrounds

	df	Ventral color			Ventral Brightness		
		F	P	Adjusted P	F	P	Adjusted P
Phenotype	1	0.30	0.582	0.582	111.08	**< 0.001**	**< 0.001**
Viewer	1	15.43	**< 0.001**	**< 0.001**	9.61	**0.002**	**0.003**
Background	3	35.22	**< 0.001**	**< 0.001**	31.62	**< 0.001**	**< 0.001**
Phenotype*Viewer	1	0.30	0.586	0.781	0.01	0.958	0.958
Phenotype*Background	3	2.52	*0.060*	0.091	2.43	*0.068*	0.091
Viewer*Background	3	1.97	0.120	0.240	0.51	0.673	0.673
Phenotype*Viewer*Background	3	0.04	0.987	0.999	0.01	0.999	0.999

Significant effects ($\alpha = 0.05$) are in *bold*; statistical trends are in *italics*

Fig. 5 Chromatic and achromatic visual contrasts between the ventral skin of frogs and their calling backgrounds, as seen by conspecifics and potential bird predators. Contrasts were calculated on common calling backgrounds: trunks (**a, b**), mossy rocks (**c, d**), green leaves (**e, f**), and leaf litter (**g, h**). *Error bars* show the SE of means
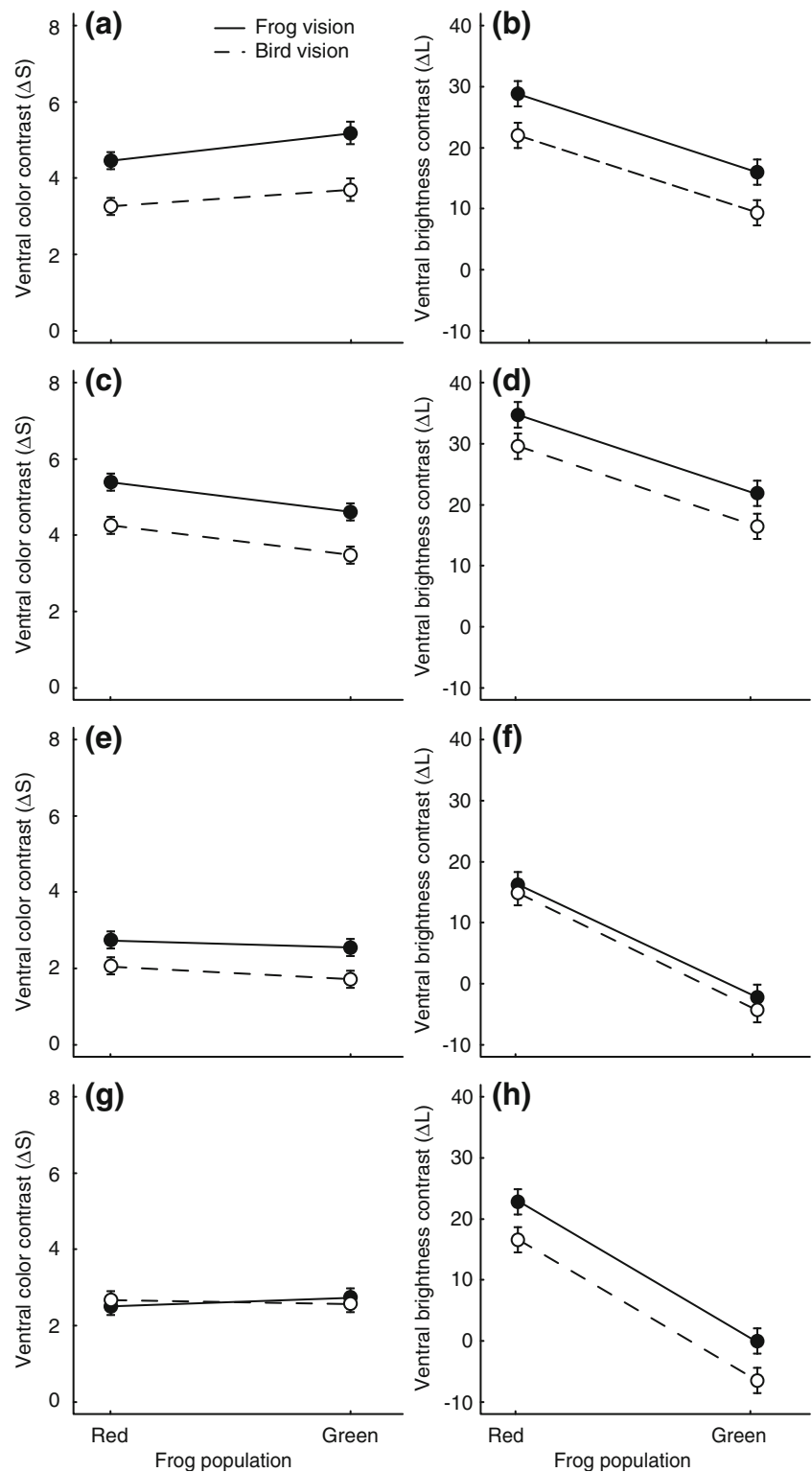
whether this difference in exposure is due to differences in frog behavior or habitat structure or both. Because of the relatively small spatial scale of our measurement (1 m), it seems unlikely that differences in exposure between color morphs are a consequence of habitat differences alone, though experiments of calling site selection in both populations would be necessary to disentangle the two possibilities. Either way, our behavioral observations suggest that green

Fig. 6 Behavior of green and red males without females (advertisement context) and in the presence of a female (courtship context): **a** mean calling activity as the proportion of time spent calling, **b** mean visual exposure (see 'Methods' section), and **c** mean height of calling position. *Bars* indicated the standard error of means
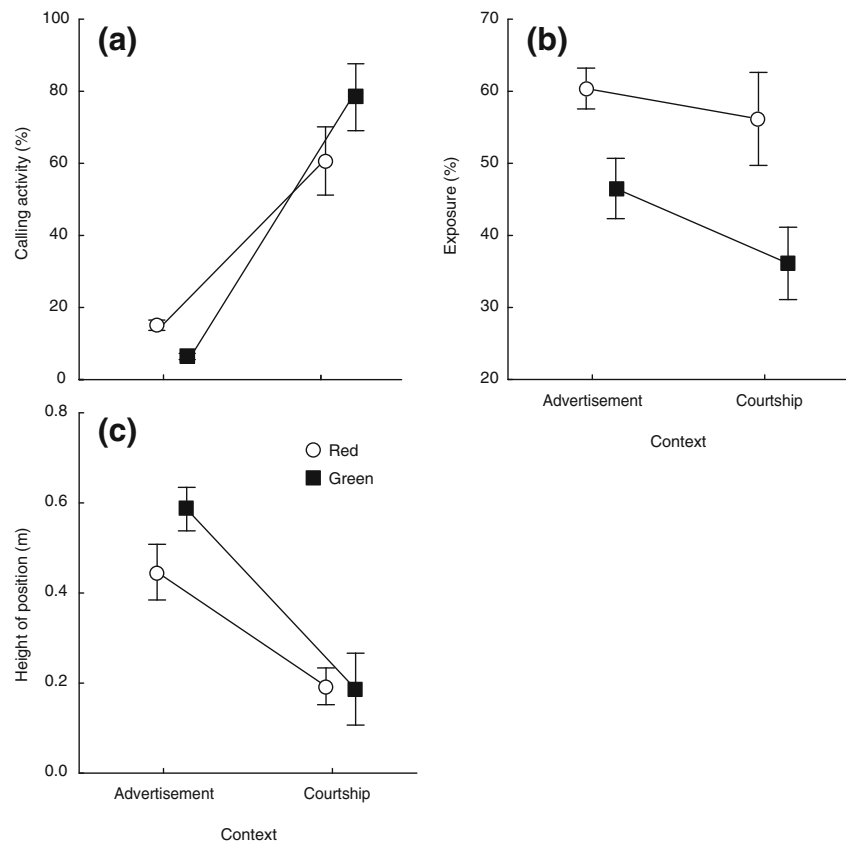
males achieve high acoustic conspicuousness when in proximity of a potential mate, but the exposure of their sexual displays appears somewhat constrained.

Other explanations, besides a trade-off between sexual and natural selection, could account for the differences in conspicuous behavior between red and green frogs. Reduced conspicuousness in terms of calling activity and other energy-demanding behaviors would also be expected if food availability is lower in the northern populations where green frogs occur. Also, reduced behavioral conspicuousness would be favored in green frogs if predation pressure is stronger or if predator composition differs and predators that cue on behavioral signals are more common in the north. To date, we know surprisingly little about ecological differences between populations of different color morphs in *O. granulifera* and other color divergent species of poison frogs. However, such ecological differences need not to be an exclusive alternative to the evolution of a behaviorally and as well as visually cryptic morph in the North, but aid explain why less conspicuous phenotypes have evolved from aposematic ancestors. Evidence that visual conspicuousness correlates with behavior of poison frogs comes from four populations in *O. granulifera* (Willink et al. 2013) and eight in *O. pumilio* (Pröhl and Ostrowski 2011; Rudh et al. 2013). These populations are ideal for further studies aimed to discover the links between ecological differences and divergence in frog conspicuousness.

General conclusions

Aposematism is assumed to evolve linked to a number of non-predation benefits like opportunities for bold foraging (Speed et al. 2009) and exaggerated sexual displays (Rudh et al. 2011), and this may explain why aposematism has evolved multiple times but there are few examples of relatively cryptic phenotypes evolving from aposematic ancestors. Apparent crypsis has evolved de novo in at least two species of poison-dart frogs (Wang and Shaffer 2008; Wang 2011), one moth (DaCosta 2010), and possibly, a toad (Bonansea and Vaira 2012). The green phenotype of *O. granulifera* seems cryptic because it has reduced visual contrast for potential predatory birds, long latency to perform an escape response, and reduced movement, foraging, and calling rates compared to the red phenotype (Willink et al. 2013).

However, red males are not much brighter from a frog's perspective and the correlation between color and behavioral conspicuousness previously described across populations (Willink et al. 2013) may disappear at times when sexual selection is strongest. In addition, toxicity is inversely correlated with visual conspicuousness in this species (Wang 2011). Thus, high toxicity in green frogs may provide protection that is particularly advantageous during courtship, when the reproductive costs of behaving cryptically are higher. Encounters between both potential predators and mates and green frogs are

probably less frequent than encounters with red males, due to their relatively cryptic appearance and behavior (Willink et al. 2013). Relatively infrequent encounters may hinder predator learning to avoid noxious prey (Roper and Wistow 1986) and reduce reproductive opportunities. However, with high toxicity, relatively high dorsal conspicuousness to conspecifics, and strikingly plastic calling behavior, green males might make the best out of these few encounters. Taken together with previous studies, our results support the notion that populations of phenotypically divergent species may use different solutions to the trade-off between natural and sexual selection, by adjusting the place and time of displays to risks and opportunities (e.g., Endler 1987). In poison frogs, this may have contributed to the dramatic variation in color pattern conspicuousness observed across species.

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