Original Article

A multifunctional warning signal behaves as an agonistic status signal in a poison frog

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Aposematic species use conspicuous "warning" signals to communicate unprofitability to potential predators. Although warning signals are classic examples of communication systems that evolved by natural selection, they can also function in the context of sexual communication and are therefore particularly useful for investigating conspicuous trait evolution under multifarious selection. To test whether aposematic signals also serve to mediate intrasexual disputes, we observed males from a highly territorial poison frog species (*Oophaga pumilio*) in their native territories and in experimental dyadic contests to assess the influences of body characteristics such as warning signal brightness and body size on the outcomes of territorial interactions. We report here that although neither male size (snout–vent length) nor mass significantly predicted male aggressiveness (latency to call) in dyadic contests, a male's dorsal brightness was a significant predictor of willingness to initiate aggressive interactions, with brighter males exhibiting a shorter latency to call than duller males. Furthermore, brightness asymmetries between males predicted the outcomes of contests such that asymmetries were smaller in escalated aggression trials (where both males called), and brighter males were more likely to be the sole aggressor in trials with large asymmetries. These tests, combined with previous work, provide evidence that warning coloration has been co-opted as an agonistic indicator trait in this aposematic amphibian and reveal the potential evolutionary lability of conspicuous traits that arise through natural selection.

Key words: **aggression, aposematism, brightness, indicator trait, intrasexual selection, territorial signal.**

INTRODUCTION

Humans have admired and puzzled over conspicuous animal signals for millennia (Darwin 1871; Ovid 2008). The expression of these traits typically represents a compromise between the forces of natural selection and sexual selection (Darwin 1871, 1887; Endler 1983). Although sexual selection often drives the evolution of conspicuous traits (Andersson 1994), these traits can also evolve via the process of natural selection. In aposematic species, conspicuous signals such as bright "warning coloration" have evolved to communicate some form of unprofitability to predators (Wallace 1867; Ruxton et al. 2004). By virtue of their conspicuousness, warning signals may often be co-opted for use in other scenarios, such as intraspecific communication. Thus, although predators have historically been thought of as the primary agents shaping the evolution of aposematic signals (Müller 1879), evidence suggests that these signals can function in the context of mate selection (Jiggins et al. 2001; Nokelainen et al. 2012) and that sexual selection can influence the direction of aposematic trait evolution (Maan and Cummings 2009). Conspicuous sexual signals often serve as traits

of dual utility, used in mate choice and in male contests (e.g., reviewed in Berglund et al. 1996). However, despite a rapidly growing body of evidence that conspecifics pay attention to aposematic coloration in potential mates (Summers et al. 1999; Jiggins et al. 2001; Maan and Cummings 2008; Nokelainen et al. 2012), investigations into the influence of male–male competition on warning signal evolution have been rare (Crothers et al. 2011; Rudh et al. 2013).

The potential for intrasexual selection to impact warning coloration is especially probable given the well-documented role that conspicuous, nonaposematic signals play in territorial behaviors (Andersson 1994; Berglund et al. 1996). Although phenotypic characters used in agonistic assessment are often intrinsically linked to fighting ability (body size: Huntingford and Turner 1987; Riechert 2000; weapons: Emlen 2008), conspicuous coloration has evolved to function as an agonistic assessment signal (Maynard Smith et al. 1988) in some birds (e.g., "badge of status" signals: Rohwer 1975, 1982; Møller 1987; Johnstone and Norris 1993; Pryke et al. 2001; Alonso-Alvarez et al. 2004; reviewed in Senar 2006), lizards (e.g., Stapley and Whiting 2006; Whiting et al. 2006; Hamilton et al. 2013), fish (Siebeck 2004), and insects (e.g., Tibbetts and Lindsay 2008). In species where territorial interactions are common, these identifiable agonistic signals can allow for the assessment of rival

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aggressiveness or resource holding potential (RHP). If asymmetries in these traits are perceptible to contest participants, then interactions can be settled before overt aggression commences (Maynard Smith et al. 1988). Because aposematic signals are often highly conspicuous, can correlate with metabolic phenotype (Santos and Cannatella 2011; Pegram et al. 2013), and may be readily co-opted as sexual signals, they represent clear candidates for agonistic indicator signals. Here, we use natural phenotypic variation in the highly territorial aposematic strawberry poison frog (*Oophaga [Dendrobates] pumilio*) to assess the influence of bright male warning coloration on aggressiveness and on the outcomes of dyadic male contests.

The strawberry poison frog exhibits dramatic variation in hue and brightness across island populations and on the mainland of the Bocas del Toro archipelago of Panama (Daly and Myers 1967). Genetic drift has largely been ruled out as a major source of this variation (Rudh et al. 2007; Brown et al. 2010; Wang and Summers 2010); sexual selection is believed to be the major force promoting warning color diversification in this species (reviewed in Cummings and Crothers 2013; Gehara et al. 2013). Female *O. pumilio* show preferences for brighter males, and at least one population contains males that are significantly brighter than females (Solarte population; Maan and Cummings 2009). *Oophaga pumilio* is characterized by elaborate maternal care (Summers et al. 1997) and great variance in male mating success (Pröhl and Hodl 1999). Furthermore, males of the species are highly territorial, exhibiting territory site fidelity (McVey et al. 1981), and guarding areas that contain sites for foraging, tadpole rearing, and perches for calling to females (Donnelly 1989; Pröhl 1997). Male *O. pumilio* maintain small territories, which in dense populations such as Solarte average only 2–3 m^2 (Pröhl and Ostrowski 2011), and which they vigorously defend against each other through vocalizations and close-range aggressive encounters (Bunnell 1973; Forester et al. 1993; Baugh and Forester 1994; Gardner and Graves 2005; Pröhl 2005).

Hence, sexual selection in this species appears to have significant intersexual and intrasexual components, and the exceptionally bright warning coloration found in males of the Solarte population appears likely to be used in competitive interactions (Crothers et al. 2011). Here, we test whether the brightness of males' warning coloration can be used as an indicator of aggressiveness or competitive ability in this population of an aposematic species, a possibility that until now has been unexplored in any aposematic animal. Although variation in hue and brightness are correlated traits in this population (Maan and Cummings 2009; Crothers and Cummings 2013), laboratory experiments that independently manipulate the brightness component of male signals show that both females (Maan and Cummings 2009) and males (Crothers et al. 2011) attend to variation in male brightness. Furthermore, visual modeling efforts suggest that the natural variation in male dorsal brightness in this population is likely to be more visually detectable to conspecifics than concomitant variation in dorsal hue or color (Crothers and Cummings 2013). Hence, we focus on male brightness and explore whether the natural variation in male dorsal brightness in this population is predictive of male aggressive behavior, by testing these behaviors in staged agonistic encounters and in simulated territorial intrusions in the field.

METHODS

Territorial adult males were located in the field during daytime hours in 2010 and 2012 on Isla Solarte, in Bocas del Toro, Panama (09°20.014′N, 82°13.197′W). Males were captured and kept individually in plastic 475-mL deli containers moistened with UV-purified water until body measurements were taken within several hours of capture at the Smithsonian Tropical Research Institute (STRI) in Bocas del Toro, Panama. In total, we assessed the behavior of 110 males in staged dyadic contests and a different set of 109 males in field experiments that simulated territorial intrusions using acoustic playbacks (field territorial tests) following approved Institutional Animal Care and Use Committee (IACUC) protocols (UT AUP-2010-00139).

Staged dyadic contests

In June and July of 2010, we conducted a series of behavioral tests in the field to elucidate the intrinsic competitive abilities of males of varying brightness. One hundred ten calling males were captured in their territories and immediately placed in a dyadic contest paradigm that allowed for unrestricted contact between frogs. Males were paired opportunistically as soon as they were captured, for a total of 55 fighting trials, and later measured for body characteristics (within several hours of capture). To eliminate the possible influence of prior testing on a male's motivation to interact with conspecifics, no male was tested more than once. For a subset of trials, we measured male body temperature both before the trial began and immediately afterward ($N = 52$ males; see Body Measurements).

Males were allowed to acclimate for 5 min under clear enclosures on opposite ends of a clear acrylic neutral arena ($L = 61$ cm, $W = 20$ cm, $H = 20$ cm) in the field. After 5 min, the acclimation enclosures were removed and the males were allowed to interact freely for 15 min. During the interaction period, observers scored frequencies of common aggressive behaviors (Table 1; following Baugh and Forester 1994), as well as latency to initiate calling, which was used as a proxy for territorial motivation and aggressiveness.

Field territoriality tests

To test whether male brightness correlates with his response to a simulated agonistic threat in his territory, we conducted a series of behavioral observations of 109 calling territorial males in June–July of 2012. A small speaker (Altec Lansing iM-237) was placed on the ground at a distance of 60 cm away from each male, measured horizontally from directly below the male's perch. As a visual stimulus, we placed a 3D-printed and hand-painted plastic model on top of the speaker (Figure 1; model from Turbosquid.com, printing by Shapeways, Inc.). Following speaker placement, the male was observed for 5 min to allow him to habituate to the presence of the speaker and to collect data on his baseline activity.

A recording of a male call was then broadcasted using a SanDisk Sansa Clip+ MP3 player, and the male's responses to the call were recorded for 5 min. The acoustic stimulus consisted of a 15-s-long segment of an average male call alternating with 15 s of silence. The recording of a Solarte male of average dorsal brightness and exhibiting average call characteristics (dominant frequency, call rate, call duration, duty cycle, pulse rate) out of a sample of 41 males recorded in 2009 was used as the stimulus call. The sound pressure level of the stimulus call playback was ~61 dB (dB SPL re 20 mPa), as measured 60 cm from the speaker in the field using a Pyle PSPL01 Mini digital sound level meter. During the playback, we focused on behaviors that could unambiguously be assigned as responses to the playback rather than nearby conspecifics, including the male's latency to orient to the speaker (male turns body to face

Table 1

Behaviors scored during staged dyadic contests

Behavior	Description
Call	Male orients body toward other frog and inflates vocal pouch while producing a rapid burst of chirps
Track	Male orients body toward other frog without moving forward
Approach	Male orients body toward other frog while moving forward
Move away	Male orients body away from other frog while moving
Charge	Male runs toward the other frog and veers away without making physical contact or makes physical contact with the other frog
Grapple	Male seizes other frog's limbs
Pin	Male sits on other frog or wraps body around the dorsal surface of other frog
Chase	Male pursues other frog while his opponent retreats
Escape	In response to aggression from other frog, male rapidly moves away from aggressor toward a distant side of the test chamber
Statue	In response to aggressive behavior from other male, the submissive frog remains frozen in one position

Behaviors are a modified version of those described in Baugh and Forester (1994).
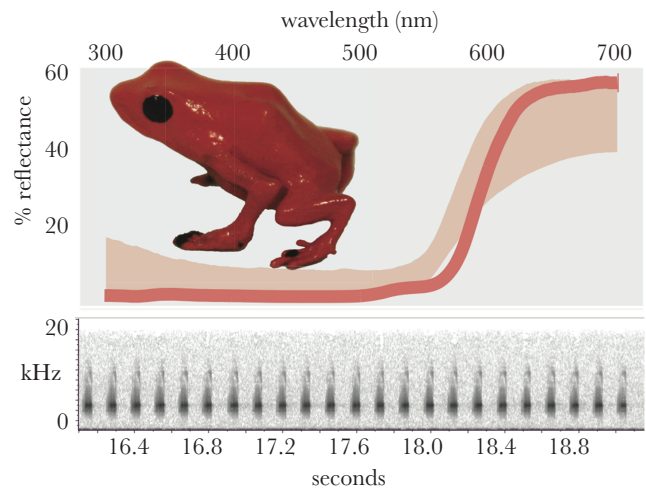

Figure 1

Photograph and spectral plot of plastic model and spectrogram of acoustic stimulus used in playback experiments. The red line on the spectral plot depicts the reflectance of the plastic stimulus model across the measured wavelengths and is bounded by the reflectance of the dullest and brightest males of a sample of 139 males measured in 2012 (pale tan area of plot).

in the direction of the speaker), whether the male approached the speaker (within a distance of 10 cm or less), and whether the male interacted with the model frog (oriented body toward model and called while standing on the speaker, made contact with the model, or tackled the model).

Body measurements

Males were taken to STRI following behavioral observation and measured on the day of capture. All males were measured for body length (snout–vent length or SVL), mass (to the nearest 0.01 g), spectral reflectance, body temperature at the dorsal surface (within 0.1 °C), and were photographed on a standard background against a ruler. Body temperature was measured using an infrared laser thermometer (Mastercool, Randolph, NJ) immediately prior to spectral reflectance measurements for all frogs. In 2010, SVL was measured from photographs using ImageJ software (Rasband 1997–2014) and with manual calipers. SVL was measured using only digital calipers in 2012. In 2010, because photographs provided more accurate and precise body length estimates than those taken with calipers, we used the ImageJ measurements for these analyses. Eleven frogs were not photographed using standard photograph conditions in 2010; thus, a data set of 138 males from this

population measured during that field season was used to impute missing ImageJ SVL values using *k*-nearest neighbor averaging of caliper/photographed SVL measurements using the R imputation package (Wong 2013).

Spectral reflectance measurements were taken at the head and dorsum (2 measurements per region in 2010 and 4 in 2012) using an EPP2000 UV-VIS portable spectrometer and R600-8 UV-VIS-SR reflectance probe (StellarNet Inc., Tampa, FL) and a PX2 Xenon flash lamp outfitted with a custom-made 50-Hz trigger input (Ocean Optics, Dunedin, FL). Spectralon white standard measurements were taken frequently to account for lamp drift. Dorsal reflectance spectra were obtained by averaging dorsal reflectance measurements of the head and dorsum. Averaged dorsal reflectance spectra were used to calculate the total reflectance flux (referred to in text as "brightness": $[\sum_{300\,nm}^{700\,nm} R(\lambda)]$), a perceptually unbiased estimate of male brightness.

Statistical analysis

All statistics were performed in R 2.15.1 (R Development Core Team 2012). Correlations among predictor variables violate the assumptions underlying the statistical models described below. Brightness and SVL were weakly positively correlated in the 2010 dyadic contest data set ($N = 110$, $P = 0.017$, $r = 0.22$); both traits were included in those models to isolate their independent effects. SVL and mass were positively correlated in the 2012 data set ($N = 94$; $P < 0.0001$, $r = 0.54$), we, therefore, only used SVL and brightness as male traits in those models. Significance of model predictor variables was assessed using Wald and likelihood-ratio chi-square statistics calculated from Type II analysis of deviance/variance tests in the car package (Fox and Weisberg 2011). Significance of overall models was assessed by comparisons to those fitted with only an intercept term.

Hypothesis testing in staged dyadic contests

We tested 2 hypotheses concerning male brightness and aggression: 1) a male's brightness predicts his own readiness to initiate territorial interactions and 2) asymmetries in brightness between males predict the outcomes of dyadic contests. Calls are a central component of agonistic interactions between *O. pumilio* males (Baugh and Forester 1994; Pröhl 2005). Calling behavior also appears to be a reliable predictor of dominance outcomes in *O. pumilio* (Baugh and Forester 1994) and in *Eleutherodactylus coqui*, another small Neotropical frog (Stewart and Rand 1991). Therefore, we assessed whether brighter males more readily initiated aggressive interactions in these trials by calling.

Of the 51 males that called, the latency for a male to start calling was modeled using a multivariable generalized estimating equation (GEE), using Poisson distribution with log link and an exchangeable correlation structure in the geepack package, and including male brightness, SVL, and mass as covariates (Højsgaard et al. 2006). This type of model produces estimates similar to those of generalized linear models, but with the estimated variances adjusted for the correlation of behavioral outcomes within each male pair, while also being permissive to violations of the distributional assumptions of mixed models (Zuur et al. 2009; Hardin and Hilbe 2012).

We next sought to determine whether trait asymmetries between paired males impacted the level of aggression/interaction exhibited in those trials, as a male's behavior will likely be influenced by the size of his rival's traits relative to his own (Enquist et al. 1990). Asymmetries in male traits were calculated by taking the absolute value of the logged ratio in trait values between the 2 males in a given trial (calculation is equivalent to that calculated in Enquist et al. 1990):

$$= | \log_{10}(\text{Value}_{\text{Male1}} / \text{Value}_{\text{Male2}}) |$$

These asymmetries were calculated for brightness, SVL, and mass and were included as covariates in the models described below.

Only 11 out of the 55 trials involved highly aggressive behaviors such as charges, grapples, pinning of the other male, and chases, consistent with past studies of *O. pumilio* behavior (Pröhl and Berke 2001). We first used calling behavior to assess the level of aggression exhibited by the pair, coded as an ordinal response variable (no males called, one frog in the pair called, or both called), and modeled using ordinal logistic regression with the polr function in the MASS package (Venables and Ripley 2002). We also used Wilcoxon rank sum tests to determine whether trait asymmetries differed in escalated aggression trials where both males called (8 out of 55 trials) versus less aggressive trials where only one or none of the frogs called (47 trials).

In many taxa that have been studied, initiators are often the winners of agonistic interactions (e.g., Bekoff and Scott 1989; Jackson 1991; Stewart and Rand 1991; Hsu et al. 2009). We, therefore, performed a chi-square goodness-of-fit test, testing the hypothesis that the initiator (first male to call out of the 43 trials where males called) of an interaction was equally likely to rank above or below his rival in terms of brightness, SVL, or mass. To assess how relative male body length impacted the outcomes of these trials, we used a binomial generalized linear model (GLM) to determine whether body length asymmetries between males predicted whether the brighter male of a pair was more likely to be the initiator. We then limited our analyses to only the 19 of these trials where males were approximately matched for body length (<1 standard deviation [SD] difference) and used a chi-square goodness-of-fit test to see if the brighter male of these pairings was more likely to initiate aggression. Furthermore, in the majority of contests (35 out of 55), only one male was aggressive (=called). We, therefore, tested whether trait asymmetries influenced the likelihood that the brighter or duller male of the pair was the sole aggressor, using a binomial GLM with logit link.

Finally, we assessed the influence of trait asymmetries on the likelihood of a trial resulting in the most frequently documented highly aggressive behavior (charges: 9/55 trials) using a binomial GLM. We explored this question both by calculating the trait asymmetries as described above and by classifying males into brightness categories (brighter than the mean for the data set; duller than the mean). In the second case, we predicted that males classified in the

same brightness category (both brighter than average or both duller than average) might be more likely to escalate and show highly aggressive behaviors than males falling into disparate categories.

Male body measurements were taken within several hours of the dyadic behavior tests. As an indirect way of assessing whether the outcomes of our tests impacted the brightness of the males, we performed 2 analyses focusing on body temperature changes in the males. Amphibians are ectothermic, so changes in an animal's body temperature can provide some information about the metabolic costs of these trials. We first compared the change in body temperature between 3 types of frogs—1) initiators, 2) noninitiators, and 3) frogs in trials where no males called—using a Kruskal–Wallis rank sum test ($N = 52$ males). We also tested whether the brightness of males in trials where males were highly aggressive and charged at one another ($N = 9$ trials) differed from the brightness of males in trials involving no charges ($N = 46$ trials; Wilcoxon rank sum test).

Hypothesis testing in field territoriality tests

By testing male aggressive response to a simulated territorial intrusion in naturalistic settings, we tested 2 additional hypotheses regarding male brightness: 1) brighter males more readily respond to an intruder in their territory and 2) brighter males are more likely to be interacting with conspecifics (as predicted by Maan and Cummings 2009; Crothers et al. 2011). Fifteen of the 109 males observed in the playback experiments had been previously captured earlier in the field season (as identified by distinct toe clippings found on frogs after behavior observations) and were removed from the analysis to ensure no accidental retesting. Twenty males were calling to nearby conspecifics (<60 cm away) during their baseline observation period; this was coded as a dichotomous variable and included in the analyses to account for any effect of a conspecific's presence on male response to the playback.

We first investigated whether a male's body characteristics were correlated with the probability that he was associating with a conspecific by fitting a binomial GLM with male brightness and SVL as covariates. Next, the latency for a male to orient to the model (for the 67 males that oriented) was fitted with a Poisson GLM including brightness and SVL as covariates, and correcting standard errors for overdispersion using a quasi-model where the variance is given by $\phi \times \mu$, where μ is the mean and ϕ is the dispersion parameter. Whether a male approached the speaker was coded as a dichotomous variable and modeled using a binomial GLM with brightness, SVL, and initial perch height, and whether a male was associating with a conspecific during the baseline included as covariates. Finally, very few males ($N = 9$) climbed onto the speaker and interacted with the model frog, thus we used an exact binomial test to see whether a greater number of males exceeding the average brightness or SVL attacked the model than expected by chance.

RESULTS
Staged dyadic contests
Effects of male brightness on behavior
For the staged dyadic contests, we found that 51 of the 110 males called in 43 of the 55 dyadic trials. In the 43 trials with calling males, we observed 8 trials where both males called and 35 trials where only a single male called. Brighter males called significantly faster than duller males (Figure 2a; $N = 51$; Poisson GEE; overall model $P = 0.007$; Wald$_{\text{Brightness}} = 4.02$, $P_{\text{Brightness}} = 0.045$). Male SVL and mass had no effect on call latency (Figure 2b,c; Wald $= 2.00_{\text{SVL}}$, 0.00_{Mass}, $P = 0.157_{\text{SVL}}$, 0.995_{Mass}).

Effects of brightness asymmetries between males on behavior

Interactions often involved only a handful of calls (median = 4 calls). Across all of the trials, the magnitude of the asymmetry in brightness, SVL, and mass had no impact on whether none, one, or both frogs called in a given pair (N = 55 trials; ordinal logistic regression; overall model P = 0.470; χ^2 = 1.40$_{Brightness}$, 1.04$_{SVL}$, 0.34$_{Mass}$, all Ps > 0.24). However, the brightness asymmetry between males was significantly smaller in the 8 trials where both males called (escalating) versus less aggressive (de-escalating) trials where only one or none of the males called (Figure 3a; Wilcoxon rank sum test: N = 55 trials, W = 103, P = 0.042), whereas there was no significant effect of SVL or mass asymmetry on these outcomes (Figure 3b,c; Wilcoxon rank sum tests—SVL: W = 198, P = 0.821 and mass: W = 235, P = 0.267).

Males that initiated interactions within a pair were not equally distributed among the brightness, SVL, and mass categories (in the N = 43 trials where one or more males called; chi-square contingency table test, χ^2 = 16, degrees of freedom [df] = 7, P = 0.025). Initiator males were more often the brighter, heavier, and longer of the pair than expected by chance (Supplementary Table). The magnitude of the body length asymmetry between males in these trials did not predict whether the brighter male of a pair was the initiator (binomial GLM; N = 43; z = −1.663, P = 0.096). When our analysis was limited solely to the 19 of these trials where males were matched for body length (difference < 1 SD), the brighter male of the pair was significantly more likely to initiate than the duller male (N = 14 trials with brighter initiator, 5 with duller; chi-square goodness-of-fit test: χ^2 = 4.26, df = 1, P = 0.039).

In 35 trials, there was only one aggressor (=only one male called); the magnitude of the brightness asymmetry between the frogs had a significant positive impact on the probability of the brighter male of the pair being the aggressor in these trials, whereas SVL asymmetry had a significant negative impact (Figure 4; binomial GLM; overall model
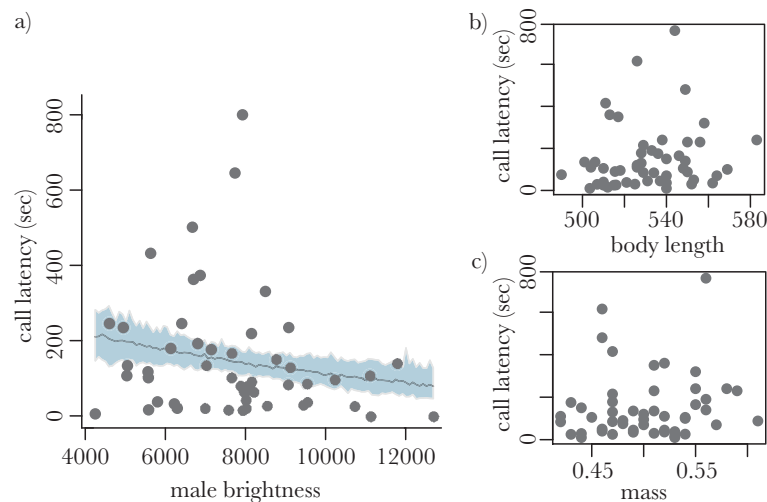

Figure 2

Relationship between males' latency to call and (a) male brightness ($\sum_{300\,nm}^{700\,nm} R(\lambda)$), (b) body length (in arbitrary units), and (c) mass (g). Dots represent data points, and the black line and shaded area represent the predicted line and 95% confidence intervals of the GEE model, respectively.
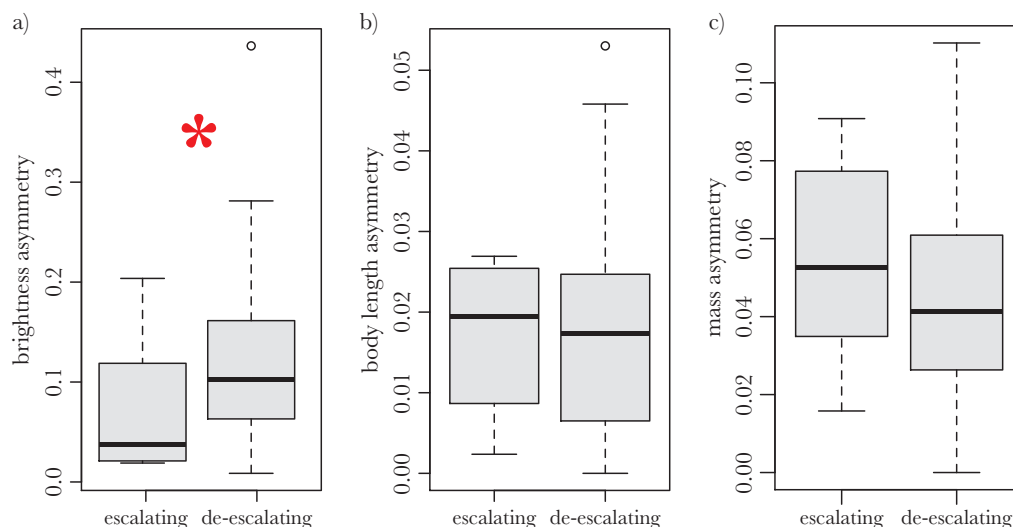

Figure 3

Box-and-whisker plots of (a) brightness asymmetry, (b) body length asymmetry, and (c) mass asymmetry between males in a given trial according to whether the trial was escalating (both males called in the trial) or de-escalating (only one male called or no males called). Boxes span the first and third quartile of the data, and horizontal black lines represent the median. Whiskers span the range of the data, excepting outliers (open circles). Asterisk indicates that the Wilcoxon rank sum test was significant (P < 0.05).

$P = 0.035$; Wald $= 4.07_{\text{Brightness}}$, 3.97_{SVL}, 1.60_{Mass}, $P = 0.044_{\text{Brightness}}$, 0.046_{SVL}, 0.206_{Mass}). Finally, trait asymmetries did not have an effect on the probability of males charging one another in a trial ($N = 55$ trials, 9 of which had charges; binomial GLM: Wald $= 2.086_{\text{Brightness}}$, 0.070_{SVL}, 0.045_{Mass}, $P = 0.15_{\text{Brightness}}$, 0.79_{SVL}, 0.83_{Mass}). However, our categorical brightness analysis revealed that charges were more likely to occur in trials where males were both ranked in the same brightness category than in trials where males were mismatched in brightness rank (binomial GLM; Wald $= 4.81$; $P = 0.028$).
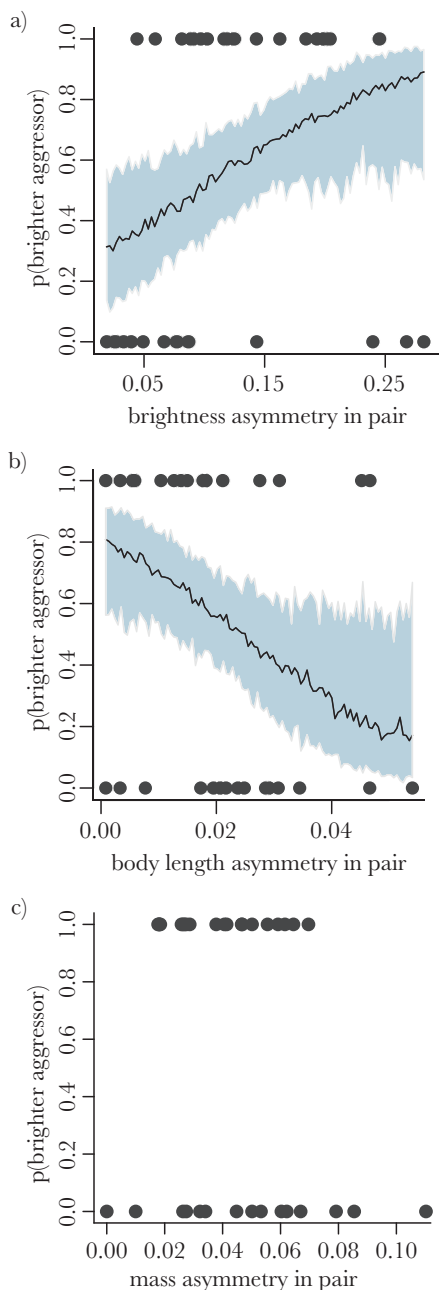

Figure 4
Probability of the brighter male of a pairing being the aggressor (in trials where only one male called) as a function of (a) the brightness asymmetry between the males, (b) the body length asymmetry between the males, and (c) the mass asymmetry between the males. Dots represent data points, and the black line and shaded area represent the predicted line and 95% confidence intervals of the binomial GLM model, respectively.

Male body measurements were taken within several hours of the dyadic behavior tests, so we cannot entirely exclude the possibility that the outcomes of our tests impacted the brightness of the males. However, very few of these interactions involved energetically costly behaviors such as protracted calling bouts or bodily contact. And when we compared the change in body temperature between 3 types of frogs—1) initiators, 2) noninitiators, and 3) frogs in trials where no males called—we found no difference between the 3 groups (Kruskal–Wallis rank sum test; $\chi^2 = 1.83$, df $= 2$, $P = 0.401$). Furthermore, the brightness of males in trials where males were highly aggressive and charged at one another ($N = 9$ trials) did not differ from the brightness of males in trials involving no charges ($N = 46$ trials; Wilcoxon rank sum test; $W = 922$, $P = 0.45$).

Field territoriality tests

There was no relationship between a male's brightness or SVL and whether he was interacting with a conspecific before the playback test (binomial GLM: $N = 94$, Wald $= 0.207_{\text{Brightness}}$, 0.447_{SVL}, $P = 0.65_{\text{Brightness}}$, 0.50_{SVL}). Brighter males oriented to the speaker significantly faster than duller males, and there was no significant effect of SVL; however, the overall model was only significant when brightness was included as the single covariate (Figure 5; $N = 67$; "quasi-Poisson" GLM: $F = 5.39$, $P = 0.023$). There was a marginally significant positive effect of male perch height on the probability of a male approaching the speaker ($N = 94$; binomial GLM, Wald $= 3.72$, $P = 0.054$), but no effect of brightness, SVL, or presence of a conspecific on this behavior (all Wald < 0.81, all Ps > 0.370). Finally, 6 out of the 9 males that interacted with the model frog were brighter than average males, and 7 were longer than average males; these results did not significantly differ from chance (exact binomial tests: $P_{\text{Brightness}} = 0.508$; $P_{\text{SVL}} = 0.180$).

DISCUSSION

Outcomes of agonistic contests are often predicted by asymmetries in male resource value (Maynard Smith and Parker 1976; e.g., residency: Shuster 1992; McMann 1993; Mohamad et al. 2012) or RHP (Maynard Smith 1982). Within this latter group of contests, aggressive interactions are often settled through comparisons of
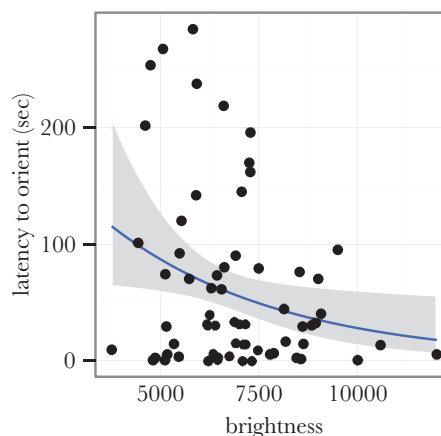

Figure 5
Relationship between male brightness and latency to orient to speaker (turning body to face speaker) during simulated territorial challenge trials. Line and shaded area flanking the line represent the predicted line and smoothed 95% confidence intervals of the "quasi-Poisson" GLM, respectively, whereas dots represent data points.

phenotypic traits that provide information on asymmetries in body size (Maynard Smith 1974; Davies and Halliday 1978; Huntingford and Turner 1987), weapons (Emlen 2008), physiological (Marden and Rollins 1994; Zamudio et al. 1995), or motivational states (e.g., Enquist and Leimar 1987; Kotahio et al. 1999; Hofmann and Schildberger 2001). Although often the phenotypic trait under comparison is directly linked to RHP (body size; weapon size), other times the trait under assessment has an indirect relationship to RHP—such as brightness (Whiting et al. 2006) or color (Rohwer 1975; Alonso-Alvarez et al. 2004; Stapley and Whiting 2006). In this aposematic frog species, the brightness of a male's conspicuous orange-colored dorsum was a more reliable indicator of a male's willingness to initiate aggressive interactions than standard phenotypic characters of male size (SVL or mass). Assessment signals can serve to counter the costs of agonistic interactions by settling the interactions prior to physical contact. Studies across taxa including red deer (Clutton-Brock and Albon 1979), cichlids (Enquist et al. 1990), field crickets (Hofmann and Schildberger 2001), and wolf spiders (Kotahio et al. 1999) show that the majority of territorial disputes are settled via noncontact phenotypic comparisons, whereas fewer disputes are settled via contact interactions. We observed a similar pattern here with the Solarte population of strawberry poison frogs, where out of 55 dyadic encounters only 11 involved some form of physical combat (grappling, charges, etc.).

An abundance of experimental and observational studies have focused on colorful male sexual signals and their roles in settling male contests (e.g., Rohwer 1975, 1982; Møller 1987; Johnstone and Norris 1993; Pryke et al. 2001; Pryke and Griffith 2006; reviewed in Senar 2006; Whiting et al. 2006; Korzan and Fernald 2007; Tibbetts and Lindsay 2008; Hamilton et al. 2013; Ligon and McGraw 2013). A common thread to many such studies is the finding that more flamboyantly colored males are more aggressive and that male contests are settled through males' assessment of relative signal intensities (e.g., Pryke et al. 2001; Senar 2006; Hamilton et al. 2013). Here, we tested whether a similar phenomenon may occur with male warning signals, a trait that presumably arose through natural selection to communicate to predators.

We use natural variation in male traits to assess the influence of male brightness (and other body attributes) on male aggression and the outcomes of dyadic male contests. We find that brighter males are more aggressive, calling faster than their duller counterparts when confronted with a conspecific rival (Figure 2), and more readily attending to male calls within/near their territories (Figure 5); these results agree with and expand on past laboratory findings that males pay attention to rival brightness when placed in agonistic dichotomous choice trials that manipulate male perception of rival brightness (Crothers et al. 2011). Males that initiated aggression in the dyadic contests (=first callers) were significantly more likely to be both the brighter and longer male of the two in a contest (Supplementary Table), though brighter males were also more often the aggressor of the pair than expected by chance in trials where males were approximately matched for body length. Furthermore, in the >60% of trials where only one male called, the magnitude of the brightness asymmetry between the males (holding the effect of asymmetry in body size constant) predicted the odds of the aggressor being the brighter male (Figure 4a).

We also provide evidence that brightness asymmetries between males in dyadic contests can be used to settle interactions without the need for further escalation (Figures 3a and 4a), as predicted by status signal hypotheses (Rohwer 1975; Enquist et al. 1990; Maynard Smith and Harper 2003). This pattern suggests that

males are using phenotypic assessment to regulate agonistic escalation and that dorsal brightness appears to be the most reliable cue of a male's aggressive intent in this species. These observations are consistent with studies investigating conspicuous male badges in frillneck lizards (Hamilton et al. 2013) and chameleons (Ligon and McGraw 2013), where more conspicuously colored males were more likely to win fights. Finally, our results indicate that brighter males were more likely to be the aggressor in these trials when SVL asymmetries were small (Figure 4b), implying that brightness information may be relied on when body size asymmetries are difficult to perceive. Thus, this study, combined with past research (Crothers et al. 2011), shows that males respond to the brightness of rivals when controlling for other factors (such as body size and behavior) and that male brightness predicts his aggression and thus his likelihood of dominating other males.

Signal brightness can function as a reliable indicator of an individual's health, aggression, and/or foraging ability across a broad range of taxonomic groups (e.g., Hamilton and Zuk 1982; Folstad and Karter 1992; Ryan and Keddy-Hector 1992; Andersson 1994). Furthermore, warning signal brightness appears to covary with some fitness-related traits in this species (advertisement call characteristics and body temperature; Crothers et al. 2011). If male warning signal brightness is functioning as a classic indicator signal used in aggressive assessment in *O. pumilio*, we would predict that contests with larger asymmetries in brightness should be less aggressive and contests with small asymmetries in brightness should reach a high state of aggressiveness (Enquist et al. 1990; Maynard Smith and Harper 2003). In our study, the level of brightness asymmetry did not correlate with whether males charged one another in a trial ($N = 9$ trials). However, we found some evidence that supported a role of brightness as an agonistic signaling trait, in that brightness asymmetries between males were smaller in escalated aggression trials (where both competitors called; Figure 3a), and brighter males were more likely to be the sole aggressor in trials with large brightness asymmetries (Figure 4a). Thus, our results indicate that warning signal brightness correlates with aggressiveness and can be used as a cue during aggressive interactions and provide some evidence that its pattern of influence on these interactions is consistent with an agonistic assessment mechanism.

Our findings indicate that brighter males may be greater territorial threats than duller males, and because they are also likely to be more noticeable by virtue of their enhanced conspicuousness, they may be more readily approached by male competitors (as found by Crothers et al. 2011). Preferential aggression toward brighter males has been observed in lazuli buntings, where dull young males are able to settle in high-quality territories because they are largely ignored by brighter, older males (Greene et al. 2000). Though past laboratory studies revealed a widespread female preference for brighter males in this population of *O. pumilio* (Maan and Cummings 2009), we did not find that brighter males were more likely than duller males to have conspecifics in their territories. More comprehensive examination of male mating success in the wild is necessary to determine if brighter, more aggressive males enjoy higher reproductive success.

It is growing increasingly evident that the forces of selection are all impacting a single, quantifiable trait in poison frogs: warning coloration. Here, we add another piece to the puzzle: the apparent co-option of warning coloration as a male agonistic signal. We provide evidence that warning signal brightness in the exceptionally conspicuous Solarte population functions as an agonistic status signal, correlating with male behavior

and predicting the outcomes of male–male interactions. These results indicate that brighter males may be superior competitors, more readily obtaining and maintaining their territories. However, the correlative nature of our study precludes us from understanding what information, if any, can be gleaned from the brightness of a male's signal. Additional research that expands on the work of Crothers et al. (2011), such as artificially manipulating male brightness and quantifying its effects on aggressive interactions and determining the relationship between male brightness and body condition, will be especially fruitful. The finding that conspicuous traits that arise through natural selection can be co-opted for use as honest sexual communication signals speaks to the common trajectory of conspicuous signals in general. Nevertheless, it is not yet clear how these 2 forces interact to promote signal diversity within this species. Current research suggests that both natural selection and sexual selection impose or promote signal honesty for aposematic brightness. However, signal honesty for natural selection refers to the relationship between dorsal brightness and toxicity across the archipelago (Maan and Cummings 2012), whereas signal honesty for intrasexual selection refers to the relationship between male brightness and agonistic behaviors within the Solarte population. Future research will have to investigate how these different behavioral and physiological traits that correlate with signal brightness interact. Though indicator models of sexual selection have generally not been considered as a mechanism that drove color diversification across the populations of the archipelago, this form of sexual selection may be operating within particular populations and account for some of the signal diversification observed in this and other aposematic species. In conclusion, our finding that a warning signal functions as a status signal speaks to the evolutionary lability of aposematic traits and their utility in investigating general patterns of signal evolution.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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