ORIGINAL ARTICLE

FEMALE PREFERENCES FOR APOSEMATIC SIGNAL COMPONENTS IN A POLYMORPHIC POISON FROG

Martine E. Maan[1,2] and Molly E. Cummings[1,3]

[1]University of Texas at Austin, Integrative Biology, 1 University Station C0930, Austin Texas 78712

  [2]E-mail: m.maan@mail.utexas.edu

  [3]E-mail: mcummings@mail.utexas.edu

Aposematic signals may be subject to conflicting selective pressures from predators and conspecifics. We studied female preferences for different components of aposematic coloration in the polymorphic poison frog *Oophaga pumilio* across several phenotypically distinct populations. This frog shows striking diversity in color and pattern between geographically isolated populations in western Panama. Results indicate that male dorsal color is the most important determiner of female preferences. We did not find consistent evidence for effects of other signal components, such as spotting pattern or ventral color. Females in two populations showed assortative preferences mediated by male dorsal coloration. In a third population we found incomplete color-assortative preference behavior, with females exhibiting strong discrimination toward one novel color but not another. These results hint at a possible interaction between sexual and natural selection: female tolerance of unfamiliar coloration patterns could facilitate the establishment of novel phenotypes that are favored by other selective pressures (e.g., predator biases). Furthermore, our study suggests that specific components of the aposematic signal (i.e., dorsal color, ventral color, and spotting pattern) are affected differently by natural and sexual selection.

KEY WORDS: Bocas del Toro Archipelago, Dendrobatidae, divergence, *Oophaga pumilio*, sexual selection, warning coloration.

Animal signals often evolve in the context of intraspecific communication, driven by sexual selection through female mate choice or male–male competition. Natural selection may subsequently influence the expression of such signals, reducing ornament elaboration (Endler 1980; Zuk and Kolluru 1998), or driving a shift to signal channels that evade detection by predators and parasites (e.g., UV signals: Silberglied 1979; Cummings et al. 2003; electric signal structure: Stoddard 1999). In some cases however, signals arise due to natural selection alone, and acquire a role in intraspecific communication secondarily. Aposematic signals that communicate prey unpalatability to potential predators can follow this trajectory (e.g., Branham and Wenzel 2003). Although both natural and sexual selection may favor conspicuous aposematic color patterns, the two selection pressures may act differentially on different signal components. As a result, aposematic signaling presents a particularly rewarding model system in which to study the effects of natural and sexual selection on signal design.

Here, we study how color pattern variation affects mate choice in a poison frog. Although most attention has been focused on the role of predators in shaping aposematic signals (Ruxton et al. 2005), there are several systems in which aposematic traits are also subject to inter- or intrasexual selection (e.g., *Heliconius* butterflies; Scott 1973; Jiggins et al. 2001; Estrada and Jiggins 2008). Poison frogs (Dendrobatidae) represent the most speciose group of aposematic vertebrates (247 named species, Grant et al. 2006). About one-third of these species sequester toxic compounds from their diet of leaf litter arthropods and are aposematic

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(conspicuous and unpalatable). In contrast with the classic theoretical prediction of aposematic signal convergence (Müller 1879), intraspecific variation in color patterns is widespread in this group (Walls 1994; Hoffman and Blouin 2000).

Our model system, the strawberry poison frog *Oophaga pumilio* (recently renamed from *Dendrobates pumilio*; Grant et al. 2006), is one of the most polymorphic Dendrobatids. In most of its range from Nicaragua to Panama, *O. pumilio* shows a characteristic red/blue phenotype. However, ~15 strikingly different phenotypes, spanning the entire range of the visual spectrum, inhabit mainland and island areas in the Bocas del Toro Archipelago in northwestern Panama (Daly and Myers 1967; Myers and Daly 1983; Siddiqi et al. 2004). Although geographic isolation has probably facilitated color divergence in this region, the isolation is fairly young: all islands were connected less than 10,000 years ago (Summers et al. 1997; Anderson and Handley 2002). This is a very short time for genetic drift to result in the extreme phenotypic diversity we see today. Indeed, Summers et al. (1997) found relatively low mitochondrial DNA diversity among color morphs, a pattern that is observed also in other Dendrobatid species (Wollenberg et al. 2006; Roberts et al. 2007).

Both natural and sexual selection mechanisms provide potential explanations for aposematic polymorphism. For example, geographic variation in predator communities and/or the availability of toxic compounds may translate into variation in optimal phenotypes. Moreover, recent studies show that prey toxicity and coloration may affect predator avoidance in a nonlinear fashion, yielding several effective trait combinations (Darst et al. 2006; see also Speed and Ruxton 2007). In the present study, we explore the potential role of sexual selection in driving or maintaining aposematic polymorphism in *O. pumilio*. Strong sexual selection in this species is supported by a highly asymmetrical parental investment between the sexes (Pröhl and Hodl 1999; Summers et al. 1999): after males have attended the fertilized clutch for about one week, females carry the tadpoles to water-holding leaf axils and provide them with trophic eggs for about seven weeks until metamorphosis (Weygoldt 1980; Brust 1993). Consistent with this division of labor, courtship in *O. pumilio* is prolonged and females court with several males before mating (Pröhl and Hodl 1999; Haase and Pröhl 2002). Moreover, *O. pumilio* coexists with two other species of aposematic Dendrobatids that have not diverged in coloration, and both these species have uniparental male care (Summers et al. 1997). Finally, *O. pumilio* has color vision (Siddiqi et al. 2004) and females use male coloration as mate choice cues: unlike nocturnal frogs that rely on acoustic communication, there is accumulating evidence that male coloration affects female mate choice in *O. pumilio* (Summers et al. 1999; Reynolds and Fitzpatrick 2007). At the same time, acoustic diver-

gence is far less pronounced than color divergence in this species (Myers and Daly 1976; Pröhl et al. 2007).

The hypothesis that divergent sexual selection on male coloration has contributed to color divergence in *O. pumilio* predicts that females use male coloration as a cue for mate choice decisions. Two earlier studies, each comparing two populations at a time, have found color-assortative association preferences (Summers et al. 1999; Reynolds and Fitzpatrick 2007). However, female preferences for aposematic coloration patterns may interact with natural selection for predator avoidance. Populations of *O. pumilio* vary in two main aposematic signal components that may be subject to different selection pressures: color (hue, reflected intensity) and pattern (presence or absence and size of spots). In the present study, we therefore use a total of five different color morphs that allow us to assess how female preferences are influenced by these different signal components.

Methods
ANIMALS
Male and female *O. pumilio* were captured in the field and kept at the Bocas del Toro Field Station of the Smithsonian Tropical Research Institute, Panama. Because *O. pumilio* does not show amplexus, we increased the probability that females were receptive by collecting females in the vicinity of territorial males in the field. Frogs were housed in individual outdoor terraria and fed a diet of termites, ants, and fruit flies. Fresh water was provided daily. Frogs were measured (snout-vent length, to the nearest 0.1 mm) and weighed (to the nearest 0.01 g) within two days after capture.

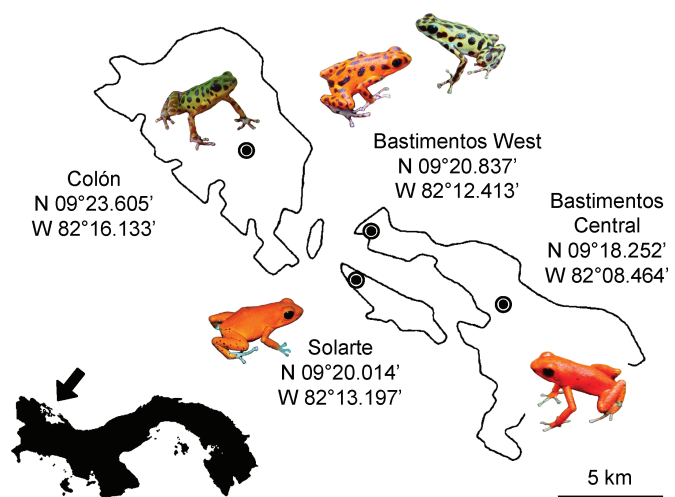We used frogs from four populations (Fig. 1, see also Summers et al. 2003):

Figure 1. Geographic locations and phenotypes of the study populations in the Bocas del Toro Archipelago in northwest Panama (see also Summers et al. 2003).

Isla Solarte (= *Cayo Nancy*)—Frogs are orange both dorsally and ventrally and generally lack spots; some individuals have tiny spots on the dorsum.

Isla Colón—Frogs are metallic green dorsally and bright yellow ventrally. All individuals have large spots on the dorsum, arms, and legs.

Isla Bastimentos—This island harbors several color morphs. We collected frogs from two distinct populations, a polymorphic population on the western tip of the island (Bastimentos West), and a monomorphic population further to the east (Bastimentos Central; Fig. 1). In the polymorphic population, all individuals have large spots, but coloration ranges from metallic green to gold to orange to red. Ventral coloration is white. For the experiments we selected green and orange individuals. In the monomorphic population, frogs look very similar to those of Solarte: they are orange dorsally and generally lack spots. Unlike Solarte frogs, Bastimentos frogs have white undersides.

MALE COLORATION

Spectral reflectance measurements were taken of each male from the head, dorsum, lower dorsum, upper throat, lower throat, and belly (two measurements per region) using a StellarNet EPP200C UV-VIS spectrometer (StellarNet Inc., Tampa, FL), SL-4 Xenon lamp, and a R400–7 reflectance probe. Spectralon white standard measurements were taken between each individual to account for lamp drift.

We calculated three summary variables for both dorsal and ventral measurements. These variables captured most of the color variation among the phenotypes used in the experiments. *Total intensity* is the total amount of reflected light in the 300–700 nm range. *LS* is derived from Endler's segment classification method (Endler 1990) and quantifies the difference between reflectance in the 600–700 nm range (orange-red) and the 400–500 nm range (blue-green) Finally, *green* is the proportion of reflectance in the 500–600 nm range relative to total reflectance.

EXPERIMENTAL TREATMENTS AND SETUP

Female preferences for males of their native population were evaluated in a series of two-way choice experiments. In all trials, a stimulus male originating from the females' native population was paired with a male from another population. To assess the role of both male coloration and spotting pattern, we conducted four different categories of experiments, characterized by the difference in color and pattern of the two stimulus males: (1) similar coloration and spotting pattern; (2) similar coloration but different spotting pattern; (3) different coloration but similar spotting pattern; and (4) different coloration and different spotting pattern. For the latter set of experiments, we also carried out control experiments in which color differences were masked (see below) to isolate differences in responses to the different coloration in stimuli from spot pattern.
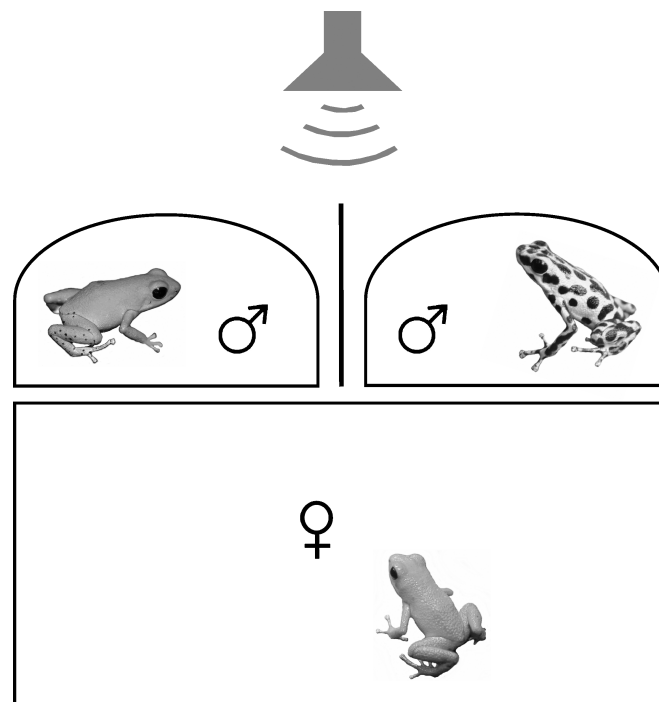

Figure 2. Mate choice experimental setup.

The experimental setup (Fig. 2) consisted of three boxes of UV-transparent clear acrylic: one female chamber (40 × 20 × 20 cm) and two male chambers (half circles with a radius of 10 cm; 20 cm high). A visual barrier blocked the males' view of each other throughout the experiment.

Previous studies (Summers et al. 1999; Reynolds and Fitzpatrick 2007) presented focal test females with other females as stimuli instead of using male stimulus frogs. Male frogs will call to females during trials and this behavior may affect female preferences, potentially interfering with the effects of male coloration. We addressed this problem in the following ways. First, we used soundproofing foam around the experimental chambers to reduce sound transmission from male to female compartments. Second, we broadcast male calls from a speaker behind the male compartments to override the sound produced by the stimulus males. Because populations differ in call properties (Pröhl et al. 2007), we used calls that were recorded in the population of the tested female. Finally, we quantified male calling behavior during trials so that we could explore its effects on female preferences statistically. This is especially important because the inflation of the vocal sac during male calling presents a visual signal that we could not control for (Narins et al. 2003). We chose to use male stimulus frogs because even though the sexes look very similar, males are smaller than females and there may be additional morphological differences that may not be noticed by humans but are meaningful to the frogs, as well as sex-specific behaviors.

Another difference with previous studies (Summers et al. 1999; Reynolds and Fitzpatrick 2007) is that instead of introducing stimuli into the female terrarium, males were presented to the test female in chambers that remained outside the female chamber. We chose this paradigm because it resembles the natural situation in which territorial males are visited by females (Pröhl and Berke 2001).

EXPERIMENTAL PROCEDURE

Male pairs were of fixed composition (i.e., within each experiment, pairs consisted of the same two individuals for each tested female) and males in a pair were matched for size and weight. We used between nine and 15 females and between three and seven male pairs in each experiment. Females were tested not more than once per day; male pairs were used for a maximum of four experiments per day.

Females acclimated to the chambers for at least 20 min before an experiment and allowed to roam freely around the experimental chamber. Male acclimation time was at least 10 min. Female vision of the males was blocked during acclimation time. After removing these visual barriers, observation started when the female approached one of the males to a distance of less than two body-lengths (i.e., "interaction zone"; within ∼4 cm). Experiments in which females did not enter the interaction zone within 15 min were discarded. Note that given the lack of amplexus in this species we have no behavioral marker for female receptivity, and likely are testing some females outside of their receptive window. The 15-min threshold for interaction should exclude females with lower receptivity. Once females had entered the interaction zone, we recorded female behavior for a period of 15 min. We recorded the time that the female spent in the interaction zone of each male and counted the following behaviors: move into interaction zone; approach male, stand against glass, and head bob. For males, we counted the number of calling bouts. To control for potential side biases, after 15 min the experimental procedure was repeated immediately with male positions reversed: the partitions were inserted again, male chambers were switched and the female was confined under the glass again for 5 sec, after which the partitions were removed and the observation started again.

Experiments were carried out in a dark room. Each male chamber was illuminated by three 75 W UV lights and one 25 W halogen light, filtered by two green-blue filters (Lee 728 + Cyan-Gel 4315) to achieve a light environment similar to that on the forest floor in the natural habitat of the frogs. In control experiments, we masked color differences using red light, provided by three 25 W ceramic red light bulbs and a combination of light filters (see Fig. 3). For orange males (Solarte and Bastimentos Central) we used two neutral density filters (Lee 182 and 298)
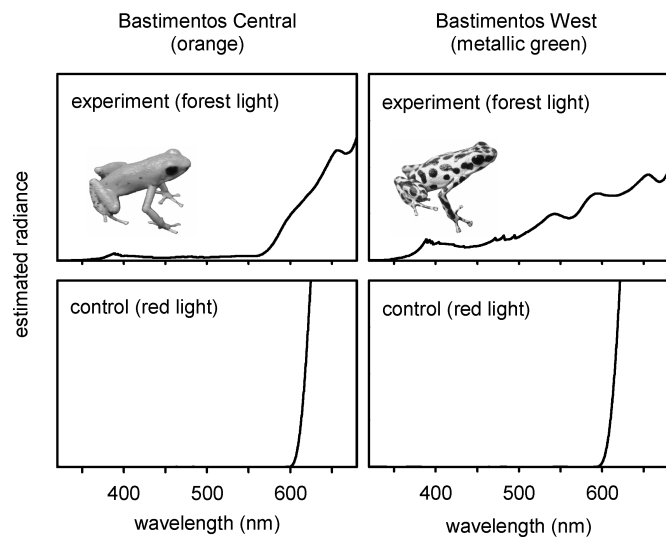

Figure 3. Dorsal radiance spectra of two males used in the mate-choice trials, illustrating the effects of the experimental light treatments. We estimated radiance with the following approximation: $L_\lambda = I_\lambda \times R_\lambda$, where I_λ is the illuminant irradiance (μEinsteins/m^2/s/sr) and R_λ is the frog reflectance (%) for a given wavelength (λ). Left panels: Bastimentos Central male (orange); right panels: Bastimentos West male (metallic green). Upper panels indicate radiance during experiments. The radiance spectrum of the orange male is dominated by longer wavelengths ($\geq$550nm); whereas the green male's spectrum is broader and richer in middle wavelengths. The total radiance of the green male is 24% higher than that of the orange male. Lower panels show radiances under the red light conditions used in controls. In these conditions, the two males show very similar radiance spectra: negligible reflectance of wavelengths below 600 nm and high reflectance beyond this point. The difference in total radiance between the males is small: the orange male reflects 2% more light than the green male. All graphs are drawn to the same scale, allowing direct comparison of the spectra.

and one red filter (Rosco 4690); for green males (Bastimentos West) we used one neutral density filter (Lee 182) and two red filters (Rosco 4690 and 4630). Illuminant radiance was quantified using the StellarNet spectrometer (StellarNet Inc.) and a 2-m fiber optic with CR-2 cosine receptor.

DATA ANALYSIS

All statistical tests were carried out in R software (R Development Core Team 2007). Female preferences were analyzed using generalized linear models (GLMs) with binomial error distribution and a logit link function. Significance was determined by F-tests examining the change in deviance following the removal of each variable. Statistics were adjusted for over- and underdispersion throughout. First, we tested whether females preferred males of their native population. Subsequently, we evaluated whether female preferences were affected by other differences between

the males in a pair (coloration, size, and calling rate). Differences between the two males in a pair were analyzed using paired *t*-tests for normally distributed data (male size and weight; color traits) and Wilcoxon signed rank tests for not normally distributed data (call rate).

Also using GLMs, we tested whether females exhibited preferences for either side of the experimental setup and whether female preferences differed between the different male pairs that we used.

Finally, we compared female preferences between experimental treatments (experiment vs. control, or females tested with different male populations) using GLMs. These models included factors for individual females because most (but not all) females were tested in both of the two treatments under comparison. Additionally, these models controlled for differences in responsiveness by including the appropriate measure of total responsiveness (i.e., total interaction time, total frequency of a behavior).

Results
MALE TRAITS
Figure 4 shows dorsal and ventral reflectance spectra of the males used in the experiments, averaged for each color morph. We address the differences between populations in the sections presenting the mate choice results. This is because in the present article we are concerned with the effects of male coloration on female choice, and color differences between stimulus males vary across male pairs. Regarding intrapopulation variation however, it is important to note here that the graphs for dorsal and ventral reflectances are drawn to different scales: for most populations, ventral coloration is brighter than dorsal coloration (paired *t*-tests, all $t > 4.732$, $P < 0.0033$; no difference for Isla Solarte: $t_{14} = 1.353$, $P = 0.20$).

FEMALE PREFERENCES
Table 1 provides an overview of the different experiments. We conducted 253 mate-choice trials, 173 (68%) of which were included in the analysis (females entered an interaction zone within 15 min after the removal of visual barriers). Females did not show significant side biases in any of the experiments or controls (paired *t*-tests: all $t < 1.54$, $P > 0.16$). Also for all trials, male calling rate did not differ between the two males in a pair (Wilcoxon tests: all $Z < 1.625$, $P > 0.10$). In one experiment we found a tendency for female preference behavior to correlate with male calling rate (see below; experiment 3a); in all other trials there were no such effects (GLMs: all $F < 2.69$, $P > 0.10$). Likewise, there were no differences in preference behavior among the different male pairs that we used (GLMs: all $F < 2.03$, $P > 0.17$), except for one experiment (see below; experiment 3c).
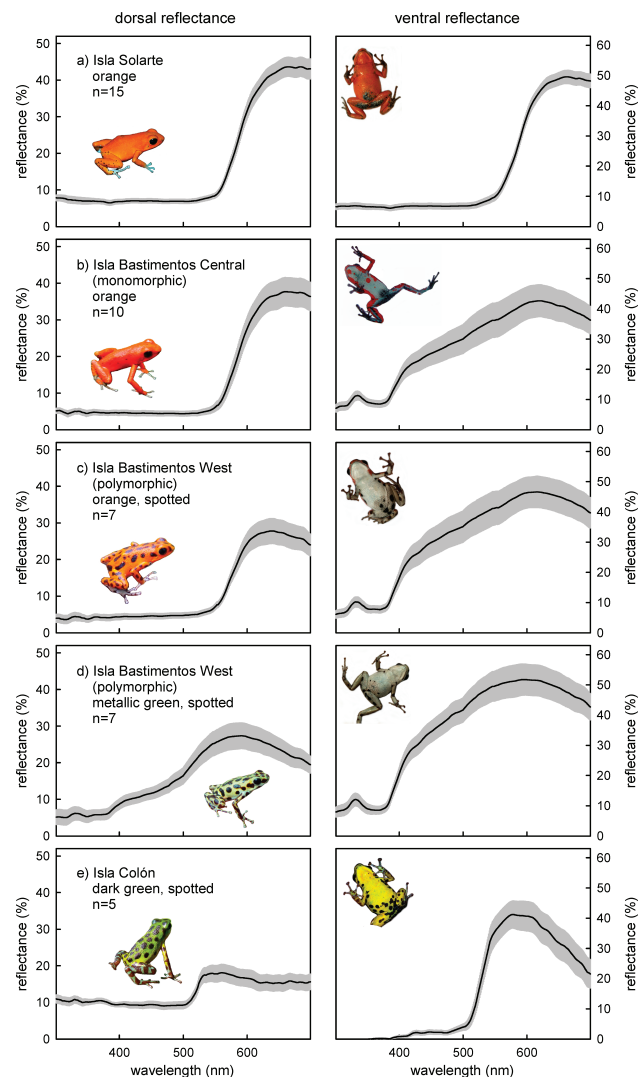

Figure 4. Male reflectance spectra. Curves represent average reflectances of all males used in the experiments. Gray areas indicate between-individual variation (standard errors). Left panels are dorsal reflectance spectra; right panels are ventral reflectance spectra.

EXPERIMENT 1: DO FEMALES SHOW POPULATION-ASSORTATIVE PREFERENCES WHEN MALES ARE SIMILAR IN DORSAL COLOR AND SPOTTING PATTERN? (FIG. 5)
Solarte females: native males (orange) versus Bastimentos Central males (monomorphic population, orange)

Solarte females did not discriminate between males from Solarte and Bastimentos Central (association time: $F_{1,16} = 0.017$, $P = 0.897$, behavior: all $F < 0.215$, $P > 0.65$). The differences in male ventral coloration (all $P < 0.02$, see Fig. 3) did not affect female preference ($F_{2,15} < 0.885$, $P > 0.433$). Likewise, whereas Solarte males tended to be larger than Bastimentos Central males (weight: $t_4 = 4.108$, $P = 0.015$; length: $t_4 = 2.43$, $P = 0.072$),

Table 1. Overview of the mate choice experiments.

Experiment 1. Female preferences when males are similar in dorsal color and spotting pattern (Fig. 5)

	females	males	result	implications
a)	Solarte	native vs. Bastimentos C	no preference	color information overrides other potential differences between populations;
b)	Bastimentos C	native vs. Solarte	no preference	male size does not affect preferences; dorsal coloration is more important than ventral coloration

Experiment 2: Female preferences when males are similar in color, but differ in spotting pattern (Fig. 6)

	females	males	result	implications
a)	Solarte	native vs. Bastimentos W orange	no preference	spotting pattern, size and ventral color do not affect preference
b)	Bastimentos C	native vs. Bastimentos W orange	preference for native males	preferences affected by male size, dorsal color or spotting pattern
c)	Bastimentos W orange	native vs. Solarte	no preference	spotting pattern, size and ventral color do not affect preference

Experiment 3: Female preferences when males differ in color, but have similar spotting pattern (Fig. 7)

	females	males	result	implications
a)	Colón	native vs. Bastimentos W green	preference for native males	preference affected by male color and male calling rate
b)	Colón	native vs. Bastimentos W orange	variable preference	preference affected by male color; variation among males affects preference

Experiment 4: Female preferences when males differ in both color and spotting pattern (Fig. 8)

	females	males	result	implications
a)	Solarte	native vs. Bastimentos W green	preference for native males	preference affected by male color (dorsal and/or ventral) and/or spotting pattern
b)	Bastimentos C	native vs. Bastimentos W green	preference for native males	

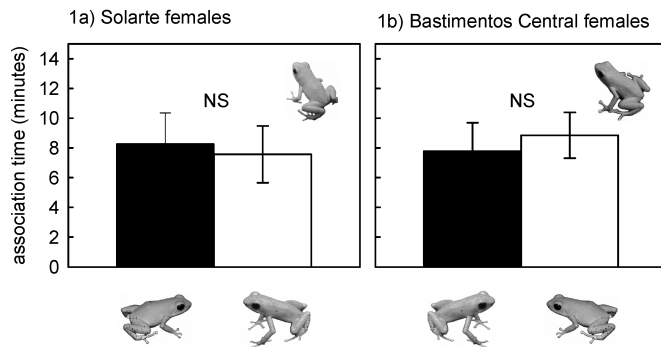Experiment 1: similar coloration, similar spotting pattern

Figure 5. Results of mate choice experiment 1, in which both hue and spotting pattern of the stimulus males were similar. Black bars indicate association time with males from the females' native population, open bars indicate association time with males from the foreign population. Error bars are standard errors. NS, not statistically significant. (A) Solarte females, choosing between native males and males from Bastimentos Central, did not show a preference. (B) Likewise, Bastimentos Central females, choosing between native males and males from Solarte, did not show a preference.

this difference did not affect female preferences ($F_{2,15} < 0.814$, $P > 0.46$).

Bastimentos Central females: native males (monomorphic population, orange) versus Solarte males (orange)

Similar to the Solarte females, Bastimentos Central females did not discriminate between males from Solarte and Bastimentos Central (association time: $F_{1,15} = 0.201, P = 0.660$). There was a tendency for females to approach the Solarte male more frequently ($F_{1,6} = 5.481, P = 0.0578$), but none of the other behaviors showed any indication of differential direction to either one of the

males (all $F < 1.341, P > 0.285$). Males differed significantly in all measures of ventral coloration (all $P < 0.01$, see Fig. 1), but this did not affect female preference ($F_{2,14} < 0.813, P > 0.463$). Likewise, although native males were smaller than Solarte males (weight: $t_7 = 5.708, P = 0.001$; length: $t_7 = 3.634, P = 0.011$), this difference did not affect female preferences ($F_{2,14} < 0.0.542$, $P > 0.593$).

EXPERIMENT 2: DO FEMALES SHOW PREFERENCES WHEN MALES ARE SIMILAR IN COLOR, BUT DIFFER IN SPOTTING PATTERN? (FIG. 6)

Solarte females: native males (orange) versus Bastimentos West males (polymorphic population, orange, spotted)

Solarte females did not discriminate between Solarte males and males from the polymorphic Bastimentos West population (association time: $F_{1,11} = 0.569, P = 0.467$; behaviors: all $F < 1.974, P > 0.210$). In the three male pairs that we used in this experiment, males did not differ in size (paired t-test, $t_2 < 1.931$, $P > 0.19$) and apart from the difference in spotting pattern, they did not differ in dorsal coloration either ($t_2 < 2.971, P > 0.097$). However, two measures of ventral coloration were significantly different between native and Bastimentos West males (LS: $t_2 = 9.249, P = 0.011$; higher in native males; and green: $t_2 < 10.7$, $P = 0.009$; higher in foreign males). None of these traits had a significant effect on female preferences (all $F_{1,11} < 3.381, P > 0.093$).

Bastimentos Central females: native males (orange) versus Bastimentos West (polymorphic population, orange, spotted)

In contrast to Solarte females, Bastimentos Central females (from the monomorphic orange population) spent significantly more

Experiment 2: similar coloration, different spotting pattern

Figure 6. Results of mate choice experiment 2, in which the spotting pattern of the stimulus males was different but the hue was similar. Black bars indicate association time with males from the females' native population, open bars indicate association time with males from the foreign population. Error bars are standard errors. NS, not significant; *, significant ($P < 0.05$). (A) Solarte females, choosing between native males and males from Bastimentos West (spotted), did not show a preference. (B) Bastimentos Central females, choosing between native males and males from Bastimentos West (spotted), spent significantly more time interacting with their native males than with the foreign males. (C) Females from Bastimentos Central, choosing between native males (spotted) and males from Bastimentos Central, did not show a preference.

time with their native males when presented with orange, spotted males from the western, polymorphic population on the same island ($F_{1,12} = 7.420$, $P = 0.019$). There were no differences in any of the behavioral measures (all $F < 3.047$, $P > 0.12$). In addition to the difference in spotting pattern, males differed in two other aspects of dorsal coloration: LS ($t_3 = 3.553$, $P = 0.038$; higher in native males) and green ($t_3 = 4.624$, $P = 0.019$; higher in foreign males). Bastimentos West males were larger than the native males (length: $t_3 = 5.524$, $P = 0.012$; weight: $t_3 = 2.994$, $P = 0.058$). After accounting for the effect of male population, none of these traits had an additional effect on female preferences (all $F_{1,11} < 1.494$, $P > 0.247$), but when modeled individually, each of these traits provided a significant predictor of female preference (all $F_{1,12} > 6.093$, $P < 0.029$). This indicates that there are several population-specific male characteristics that could have driven the observed preference for native males: male size, spotting pattern, and two measures of dorsal color.

Bastimentos West females: native males (polymorphic population, orange, spotted) versus Solarte (orange)

Females from the polymorphic population of Bastimentos West did not discriminate between their native males and the unspotted males from Solarte (association time: $F_{1,9} = 1.025$, $P = 0.338$; behaviors: all $F < 0.62$, $P > 0.46$). Except for the difference in spotting pattern, stimulus males did not differ in dorsal coloration (all $t_4 < 2.326$, $P > 0.081$). They differered significantly in all three measures of ventral coloration (all $t_4 > 3.459$, $P < 0.026$; native males reflected more light, had higher green, and lower LS than Solarte males). Native males were significantly larger than Solarte males (length: $t_4 = 4.058$, $P = 0.015$), but there was no difference in weight ($t_4 = 0.07$, $P = 0.948$). None of these differences predicted female preferences (all $F_{1,9} < 2.2106$, $P > 0.1712$).

EXPERIMENT 3: DO FEMALES SHOW PREFERENCES WHEN MALES DIFFER IN COLOR, BUT HAVE SIMILAR SPOTTING PATTERN? (FIG. 7)

Colón females: native males (dark green, spotted) versus Bastimentos West (metallic green, spotted)

When presented with a choice between their native males and green males from Bastimentos West, Colón females spent significantly more time with their native males ($F_{1,8} = 12.149$, $P = 0.008$). There were weak trends for higher frequencies of head bobs ($F_{1,4} = 5.605$, $P = 0.077$) and approaches ($F_{1,8} = 3.639$, $P = 0.093$) toward the males from the females' native population. The differences in coloration between the two males in a pair had no additional effect on female preferences (all $F_{1,8} < 0.863$, $P > 0.384$). Males did not differ in size (length and weight, $P > 0.69$). Although the difference in calling rate between males was not significant ($Z = 1.625$, $P = 0.104$), there was a tendency for

Experiment 3: different coloration, similar spotting pattern

3a) Colón females: native vs. **green** males from Bastimentos West   3b) Colón females: native vs. **orange** males from Bastimentos West
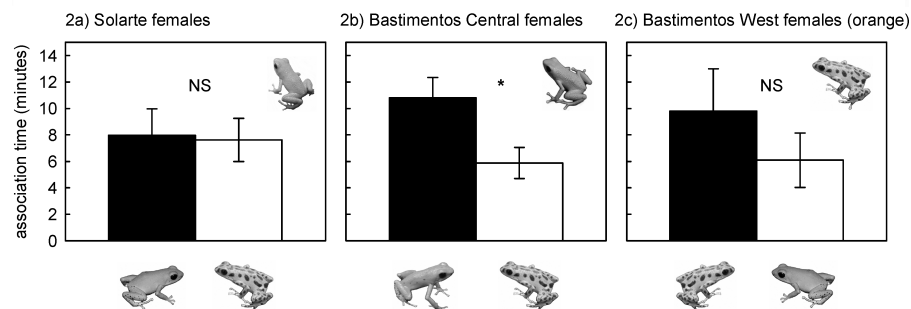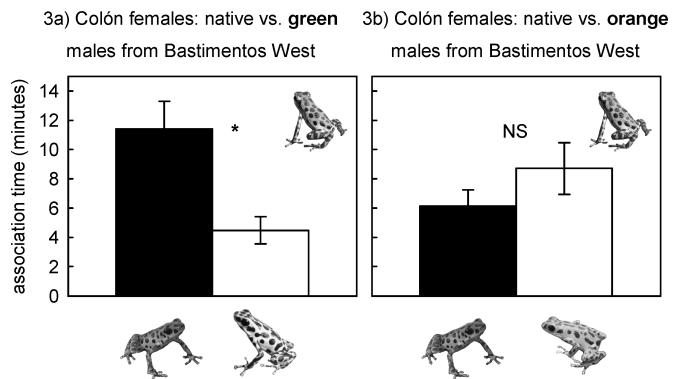
Figure 7. Results of mate choice experiment 3, in which the spotting pattern of the stimulus males was similar but the hue was different. Black bars indicate association time with males from the females' native population, open bars indicate association time with males from the foreign population. Error bars are standard errors. NS, not significant; ∗, significant ($P < 0.05$). (A) When presented with green males from Bastimentos West, Colón females spent significantly more time with their native males. (B) When presented with orange males from Bastimentos West, females showed no preference (but preferences varied significantly among male pairs, see text).

female preference to correlate with male call rate ($F_{2,7} = 4.587$, $P = 0.053$).

Colón females: native males (dark green, spotted) versus Bastimentos West (orange, spotted)

In experiments with Colón males and orange Bastimentos West males, females did not show significant preferences for either population (association time: $F_{1,13} = 1.506$, $P = 0.241$; behaviors: all $F < 0.776$, $P > 0.394$). However, females responded very differently to the three different male pairs used ($F_{2,12} = 7.552$, $P = 0.008$). One pair did not induce any preference ($n = 6$ females, that spent on average 49% of the time with the Colón male). In the second pair, females showed a tendency to spend more time with the Colón male (75%, $n = 4$, $F_{1,3} = 8.377$, $P = 0.063$). The third pair elicited a significant preference for the Bastimentos West male (females spent only 27% of the time with the Colón male, $n = 5$, $F_{1,4} = 8.082$, $P = 0.047$). Although three male pairs are insufficient to deduce which male traits determined these preference scores, two male traits correlated with female preferences: male size (females preferred Bastimentos males when they were large: $F_{2,12} = 6.713$, $P = 0.011$) and male ventral coloration (females preferred Colón males when they had high long-wavelength reflectance (LS: $F_{2,12} = 4.816$, $P = 0.029$) and low green reflectance ($F_{2,12} = 4.994$, $P = 0.026$)).

There was a trend indicating that female preference behavior differed between the two series of trials with green (3a) and orange (3b) Bastimentos West males (association time: $F_{1,8} = 4.462$, $P = 0.068$), but for behavioral measures of preference there were no

Experiment 4: different coloration, different spotting pattern

4a) Solarte females: native vs. green males from Bastimentos West
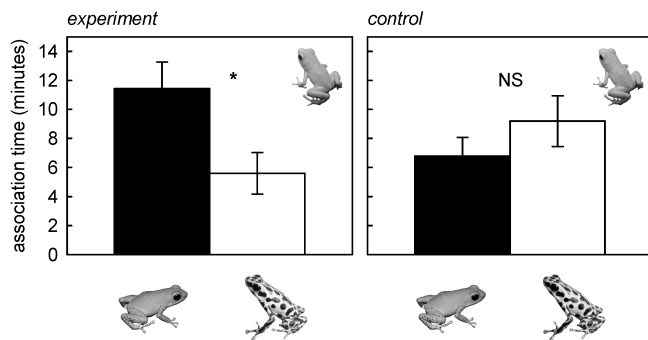
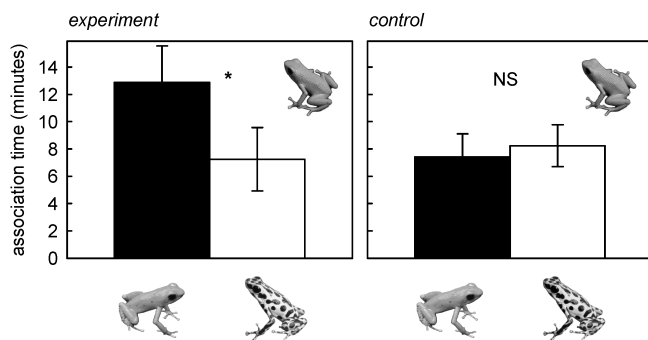4b) Bastimentos Central females: native vs. green males from Bastimentos West

Figure 8. Results of mate choice experiment 4, in which both color and spotting pattern differed between stimulus males. Black bars indicate association time with males from the females' native population, open bars indicate association time with males from the foreign population. Error bars are standard errors. NS, not significant; ∗, significant ($P < 0.05$). Left panels show the results of experiments, right panels show the results of controls in which color differences between the males were masked by red illumination. (A) Solarte females spent significantly more time interacting with native males in experiments, but not in controls. Likewise, (B) Bastimentos Central females preferred native males in experiments but not controls. For Solarte females, the difference between experiments and controls is significant.

such trends (all $F < 2.084$, $P > 0.187$). Female responsiveness did not differ between the two experiments: females spent the same amount of time in the interaction zone ($F_{1,8} = 0.732$, $P = 0.417$) and did not show different frequencies of behaviors (all $P > 0.33$).

EXPERIMENT 4: FEMALE PREFERENCES WHEN MALES DIFFER IN BOTH COLOR AND SPOTTING PATTERN (FIG. 8)

Solarte females: native males (orange) versus Bastimentos West (metallic green, spotted)

Solarte females showed significant preferences for males from their native population when presented with green, spotted males from Bastimentos West (association time: $F_{1,13} = 6.403$, $P = 0.025$). This preference was also apparent in marginally signif-

icant differences in the frequencies of several behaviors (move: $F_{1,13} = 4.557$, $P = 0.052$; stand: $F_{1,8} = 5.316$, $P = 0.050$; head bob: $F_{1,9} = 5.526$, $P = 0.043$, approach: $F_{1,9} = 4.645$, $P = 0.059$). After accounting for the effect of male population, male size and coloration did not have additional effects on female preference (all $P > 0.56$).

In control experiments under red light, masking color differences between the males (but not pattern differences), females did not display a preference (association time: $F_{1,13} = 1.284$, $P = 0.278$; behaviors: all $F < 1.125$, $P > 0.180$). Preferences measured by association time were significantly different between experiments and controls ($F_{1,11} = 18.151$, $P = 0.0013$). Total interaction time did not differ between experiments and controls ($F_{1,11} = 0.052$, $P = 0.824$). Several female behaviors occurred less frequently in controls than in experiments; after controlling for this there were significant differences in two behavioral indicators of preference (approach: $F_{1,8} = 23.460$, $P = 0.0013$; stand: $F_{1,7} = 7.751$, $P = 0.027$) and one trend (move: $F_{1,10} = 4.211$, $P = 0.067$).

A direct comparison of the responses of Solarte female toward the two Bastimentos West morphs, with either green (4a) or orange (2a) dorsal coloration, showed a statistical trend indicating stronger preferences (measured by association time) in the trials with green males ($F_{1,9} = 3.780$, $P = 0.084$).

Bastimentos females (monomorphic population, orange): native males versus Bastimentos (polymorphic, metallic green, spotted)

When presented with green males from the polymorphic Bastimentos population, females from the monomorphic Bastimentos population showed a marginally significant preference for their native males (association time: $F_{1,11} = 4.812$, $P = 0.050$). There was a trend that females approached the native male more often ($F_{1,7} = 4.702$, $P = 0.067$); other behavioral measures did not show indications for preference (all $F < 1.909$, $P > 0.195$). After accounting for the effect of male population, male size and coloration did not have additional effects on female preferences (all $P > 0.164$).

In control experiments in red light, preferences disappeared ($F_{1,12} = 0.070$, $P = 0.796$). Female interaction time tended to be lower in controls than in experiments ($F_{1,12} = 4.341$, $P = 0.059$). After controlling for this, there was no significant difference in female preferences between experiments and controls ($F_{1,11} = 0.103$, $P = 0.755$). This was also true for behavioral measures of preference (all $F < 1.605$, $P > 0.241$; absolute frequencies of behaviors did not differ between experiments and controls, all $F < 0.664$, $P > 0.431$).

Female preferences in experiments with green (4b) and orange (2b) Bastimentos males were not significantly different ($F_{1,12} = 0.042$, $P = 0.841$).

OVERALL TENDENCIES

Taking all experiments together (but excluding controls), females showed a significant overall preference for native males ($F_{1,115} = 6.158$, $P = 0.014$). We did not detect significant effects of male calling rate on female preferences in any of the experiments individually (see above), but this could be due to low incidence: males called in 27 of 136 experiments. Native males called more than nonnative males ($Z = 2.209$, $P = 0.027$). Due to the experimental design, this could also mean that males called more to native females than they did to foreign females. Among the trials in which at least one of the males called, we found a tendency that females were more likely to prefer the native male when that male called more ($\chi^2 = 2.968$, df $= 1$, $P = 0.085$).

Our experiments presented females with male pairs that differed in ventral color, dorsal color, and/or spotting pattern. To assess the effects of these different aspects of male coloration on female preferences, we conducted a GLM analysis at this level (i.e., $n = 9$ experiments, see Table 1), summarizing each of the three color components in a binomial score and using the average female association preference in each experiment as dependent variable. This analysis showed that the presence or absence of differences in dorsal color was the best, and significant, predictor of assortative preferences ($F_{1,7} = 6.642$, $P = 0.037$). Differences in ventral coloration or spotting pattern did not predict preferences (ventral color: $F_{1,7} = 0.322$, $P = 0.589$; spotting pattern: $F_{1,7} = 2.438$, $P = 0.162$).

Discussion

We investigated the role of male coloration in determining female mating preferences in the polymorphic poison frog *O. pumilio*. Together with earlier studies (Summers et al. 1999; Reynolds and Fitzpatrick 2007), our experiments present a first step toward testing the hypothesis that divergent sexual selection by female mate choice has contributed to the color diversity in this species. Moreover, we identified female preferences as well as tolerances for different components of aposematic coloration, indicating how sexual selection could interact with natural selection for predator avoidance.

Although our results confirm that male coloration affects female preferences, we show that preferences for native males strongly depend on the alternative phenotype that is presented simultaneously. Females did not discriminate between males with very similar coloration (Solarte and Bastimentos Central, experiments 1a and 1b). This finding indicates that, at least in an experimental setting, male coloration overrides other population-specific traits that could potentially influence female preferences (such as size and behavior). Given the difference in ventral color between Solarte and Bastimentos frogs (Fig. 3), the lack of preferences in this experiment also suggests that dorsal coloration

is more important than ventral coloration in determining female choice. This is consistent with the results in trials in which ventral but not dorsal color differed between males, and no significant preferences were observed (2a and 2c). Likewise, the different preferences in experiments 3a and 3b were associated with differences in dorsal rather than ventral color between the two foreign stimuli.

The finding that ventral color does not affect female preferences is surprising. In most color morphs of this species, dorsal and ventral coloration are strikingly different, with ventral colors often much brighter than dorsal colors (Fig. 4). This could be due to differential selection on these two body areas. Observations of frog behavior, in experiments as well as in the field (M. E. Maan and M. E. Cummings, pers. obs.), indicate that ventral coloration is clearly visible to interacting individuals (particularly in calling males), hinting at a role in intraspecific communication. A predator's perspective on the other hand might be dominated by a dorsal view, because the ventral surface of a walking or resting frog is close to the substrate. The hypothesis that dorsal coloration is mostly subject to natural selection and ventral coloration to sexual selection is not supported by our results: we find that of all the coloration traits measured, dorsal coloration is the most important determiner of female preferences.

Females from Isla Solarte (orange) discriminated against green males from the polymorphic Bastimentos population (experiment 4a). This is consistent with two earlier studies that showed color-assortative preferences for females from this population when presenting green stimuli from Isla Popa (Summers et al. 1999) or from Isla Colón (Reynolds and Fitzpatrick 2007). We obtained similar results for a second orange population: females from the monomorphic Bastimentos population also discriminated against green males (4b). In both cases, preferences disappeared in control experiments in which color differences were not visible.

The results from experiment 2 indicate that differences in spotting pattern may have only weak effects on female preferences. Solarte females did not discriminate against orange males from the polymorphic population on Bastimentos that, unlike Solarte frogs, have black spots (2a). In the reciprocal test, spotted females from Bastimentos did not discriminate against the unspotted males from Solarte (2c). We did find significant preferences for native males in females from the monomorphic Bastimentos population (2b). However, these preferences could have been driven by other male traits: in this experiment males also differed significantly in dorsal color and in size. Moreover, in the control experiments with green, spotted males (4b), in which color differences were masked but spots remained visible, females did not show preferences. Perhaps spotting pattern has evolved as a signal for predators rather than conspecific females. Surprisingly little is known about the relative importance of color and

pattern in determining predator avoidance, but spotting patterns may enhance conspicuousness and distinctiveness, both of which are known to promote aversion learning (Ruxton et al. 2005). Our results contrast with those of Reynolds and Fitzpatrick (2007), who found that Solarte females discriminated against Colón stimuli (green, spotted) in control trials under blue light. It is possible that this blue light treatment did not completely mask color differences. We used red light instead, ensuring that both orange and green frogs appeared equally bright (see Fig. 3).

Together, our experiments suggest that male dorsal color is the most important determiner of female preferences. We did not find consistent evidence for effects of spotting pattern, ventral coloration or male size. Male calling behavior did not significantly affect female preferences in any of the experiments, although we found a positive trend when combining all trials in which males called. A positive relationship between male calling activity and mating success has been shown for Costa Rican populations of *O. pumilio*, but it is unclear whether this was due to female mate choice, improved detection of males by females, or indirect effects of male–male competition (Pröhl 2003).

There was one experiment in which differences in dorsal color did not lead to significant female preferences: although Colón females discriminated against metallic green males from Bastimentos West (3a), they did not show overall preferences when presented with orange males from that same population (3b). However, there were significant differences in female preferences among the male pairs that we used in this latter experiment: although one male pair did not elicit any preferences, another pair elicited a preference tendency for the Colón male, and in the third pair females had a significant preference for the Bastimentos male. This indicates that variation among individual males of the same population affects female preference behavior, which has consequences for the methodology of mate-choice experiments: experimental designs should use several stimulus frogs and possibly vary the composition of male pairs. The biological implication of this result is that sexual selection may act on traits that vary between males within populations.

Sexual selection has been implicated in driving rapid divergence between geographically isolated populations in several taxa (Uy and Borgia 2000; Panhuis et al. 2001; Masta and Maddison 2002; Mendelson and Shaw 2005), including frogs (Boul et al. 2007). However, traits that mediate reproductive isolation between species or populations are not necessarily subject to sexual selection within populations (Boake 2005). Indeed, although our results confirm that male coloration influences female mate choice in *O. pumilio*, we find that this phenomenon does not always translate into population-assortative preferences (in contrast with Summers et al. 1999; Reynolds and Fitzpatrick 2007): females may tolerate or even prefer male phenotypes that deviate from the average phenotype in their native population. Ongoing

experiments are further exploring the relationship between inter- and intrasexual selection on *O. pumilio* color patterns.

Aposematic signals that function in communication with both predators and conspecifics may be subject to conflicting selective pressures. Populations of *O. pumilio* differ in several coloration traits: ventral color, dorsal color, and spotting pattern. Our experiments suggest that only dorsal color, and possibly spotting pattern, influence female mate choice. To gain a more comprehensive understanding of the evolution and maintenance of *O. pumilio* color diversity, future work will address how these different coloration components affect the avoidance behavior of predators.

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