

INTRASPECIFIC REPRODUCTIVE CHARACTER DISPLACEMENT IN A POLYMORPHIC POISON DART FROG, *DENDROBATES PUMILIO*

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Divergence in male mating signals and associated female preferences is often an important step in the process of speciation. Reproductive character displacement, the pattern of greater divergence of male signals and/or female preference in sympatry than in allopatry, has been observed in a variety of taxa with different degrees of postzygotic isolation. A number of selective processes, including reinforcement, have been proposed to cause such a pattern. Cases in which reproductive character displacement occurs among intraspecific variants are especially informative for understanding how selection acting within a species can lead to the evolution of reproductive barriers and speciation. This study tested the hypothesis that female strawberry poison dart frogs (*Dendrobates pumilio*) in polymorphic populations of the Bocas del Toro archipelago of Panama show stronger mating discrimination than do females from monomorphic populations, exhibiting an intraspecific pattern of reproductive character displacement. Our results contribute important insights into understanding selection's role in generating the striking diversity of Bocas del Toro's *D. pumilio* and provide a snapshot of what could be the early stages of reproductive isolation and speciation.

KEY WORDS: Dendrobatidae, female preference, reinforcement, sexual selection, speciation, warning coloration.

Intraspecific divergence in mate recognition systems may arise due to sexual selection (West-Eberhard 1983; Kirkpatrick 1987; Holland and Rice 1998), genetic drift (Mayr 1963), or natural selection (Fisher 1930; Dobzhansky 1937), leading to reproductive isolation among incipient species. A number of species show strong assortative mating in the absence of postzygotic incompatibilities, suggesting that divergence in mating preferences may be a common first step in the speciation process (McMillan et al. 1997; Seehausen et al. 1999; Jiggins and Mallet 2000; Jiggins et al. 2004). Reproductive character displacement (RCD)—the pattern of greater mating discrimination in sympatry than in allopatry—can occur among variants within a species (intraspecific RCD, sensu West-Eberhard 1983) as well as among

closely related species (interspecific RCD, sensu Brown and Wilson 1956). The cause of this pattern is most often attributed to reinforcement—natural selection on sexual signals or female mate preferences to avoid maladaptive hybridization (Howard 1993; Noor 1999)—although processes other than strict reinforcement have been proposed to cause RCD as well (Noor 1999; Pfennig and Pfennig 2009).

A pattern of RCD is expected to result when females that preferentially mate with males of their own phenotype produce more or fitter offspring than females who mate randomly. In this situation, selection is predicted to result in the spread and fixation of alleles that confer mating discrimination (Noor 1999). Several empirical studies have shown evidence for RCD among sympatric

taxa with incomplete postzygotic isolation (e.g., Littlejohn and Loftus-Hills 1968; Gerhardt 1994), suggesting that reinforcement contributes to the speciation process. In addition to having reduced viability or fertility, hybrids could be less fit due to inappropriate mating signals or preferences, making it difficult for them to secure mates (Coyne and Orr 1989). This type of behavioral reduction in hybrid fitness has been shown in a variety of species, including cases in which intrinsic postzygotic isolation has not been detected among the hybridizing taxa (e.g., Davies et al. 1997; Noor 1997). Such cases are especially informative for understanding how selection acting within a species can lead to the evolution of reproductive barriers and speciation.

DART FROGS OF THE BOCAS DEL TORO ARCHIPELAGO

Strawberry poison-dart frogs (*Dendrobates pumilio*, formerly *Oophaga pumilio*, see Santos et al. 2009) exhibit an amazing array of color variation on the islands of the Bocas del Toro archipelago and adjacent mainland of Panama (Daly and Myers 1967; Summers et al. 2003). This variation apparently arose with the formation of the archipelago over the past 1000–5000 years (Summers et al. 1997; Anderson and Handley 2002) and is considered to be intraspecific based on the similarity of male mating calls (Daly and Myers 1967; Prohl et al. 2007) and the fact that all populations in the archipelago (with the exception of frogs from the unrelated island of Escudo de Veraguas) fall into one clade with minimal genetic divergence in rapidly evolving molecular markers (Summers et al. 1997; Hagemann and Prohl 2007; Wang and Shaffer 2008).

Although the species is toxic (Daly and Myers 1967), and thus may have diverged in bright, aposematic coloration in response to differences in predator pressure among islands (i.e., diversifying natural selection), behavioral studies indicate that color is also important in mate choice (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008, 2009). This suggests that sexual selection may have contributed to the rapid evolution of color and pattern variation among populations (Brown et al. 2010). If mating preferences are generally strong and influenced by color, as evidence suggests (Maan and Cummings 2008, 2009), offspring from intermorph matings may have low mating success if (1) they have intermediately colored phenotypes that are outside the ranges preferred by either parental morph, or (2) the offspring themselves exhibit inappropriate mate choice preferences. The reduced fitness of these individuals could then impose indirect, diversifying selection on mating preferences, leading to further divergence through the process of reinforcement (Noor 1999; Panhuis et al. 2001; Kirkpatrick and Ravigne 2002).

Although most color and pattern variants (hereafter, color morphs) are found allopatrically, there are a few areas where two or more distinctive *D. pumilio* color morphs occur sympatrically.

The northwest tip of Isla Bastimentos is one such area (Fig. 1). Here, the dorsal background color ranges from light green to white to yellow to orange to red whereas venters are uniformly white. Within this area frogs with different dorsal coloration can frequently be found interacting and intermorph matings have been observed (C. Richards-Zawacki, pers. comm.). On the rest of Isla Bastimentos, populations are much less variable and dorsal background coloration ranges from orange to red. Nowhere on this island do we find monomorphic populations of yellow, white, or green frogs. The combination of dorsal and ventral coloration seen in these “non-red” Bastimentos phenotypes is not known to occur in any other *D. pumilio* population and their origin is unclear.

Using mate choice experiments in a laboratory setting, we tested the RCD hypothesis that females from the polymorphic area Isla Bastimentos (Bastimentos West) discriminate more strongly against potential mates with phenotypes different from their own than do females from a monomorphic area of Bastimentos (La Loma). Our results contribute important insights into understanding selection’s role in generating the striking diversity of Bocas del Toro *D. pumilio* and provide a snapshot of what could be the early stages of reproductive isolation and speciation.

Methods

FROGS

Individual *D. pumilio* were captured from two populations (A) Bastimentos West and (B) La Loma (Fig. 1) on Isla Bastimentos and housed at the Smithsonian Tropical Research Institute’s Bocas del Toro Field Station. Frogs were housed individually in plastic terraria with a substrate of soil and leaf litter. Frogs were fed vitamin-dusted termites, fruit flies, and springtails (*Collembola*) ad libitum and fresh water was provided daily. Frogs were also misted twice daily. The body size (snout-vent length, or SVL, to the nearest 0.1 mm) and weight (to the nearest 0.01 g) of each frog was measured within one day of capture. The sexes were distinguished based on throat color: mature males in these populations have dark (gray to black) throats whereas females have white throats. This method was found to reliably and repeatably distinguish males from females in these populations in a long-term mark–recapture study (C. Richards-Zawacki, unpubl. data). After the experiment, all frogs were released at their point of capture.

Frogs from the Bastimentos West population (9.3468°N, 82.2064°W, Fig. 1) are polymorphic and range in dorsal background coloration from light green to white to yellow to orange to red. This population was examined for female assortative mating in previous research (Maan and Cummings 2008), and for this current study we selected yellow and red individuals only. These colors were chosen because they were the two most abundant phenotypes in the polymorphic population. Ventral coloration is

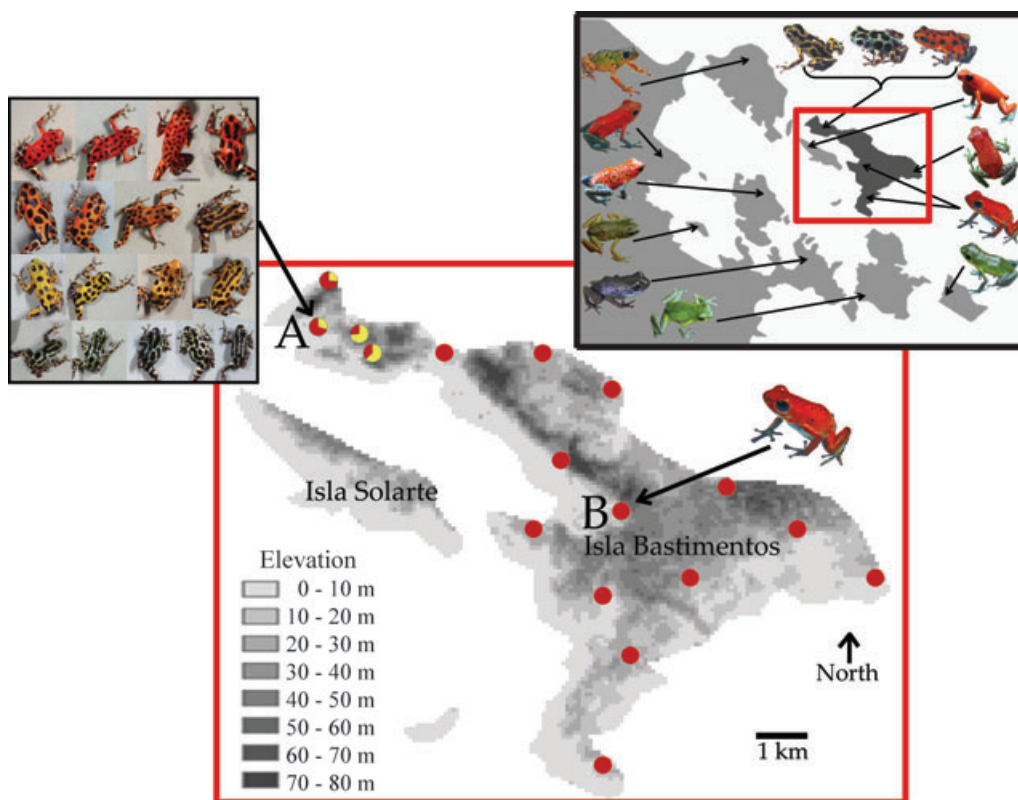


Figure 1. Map of the Bocas del Toro archipelago (upper right) showing the diversity in *D. pumilio* coloration and patterning, and inset (lower middle) showing differences in the frequency of red/orange (red) and yellow/white/light green (yellow) phenotypes among populations of *D. pumilio* on Isla Bastimentos. Locations of populations used in this study are denoted on the map (A, Bastimentos West and B, La Loma). The photos to the left show the color and pattern diversity found within the polymorphic area of Isla Bastimentos.

almost always white, although a few individuals have mottling of their predominant dorsal color on the venter and a few have one or a few ventral black spots.

Frogs from the La Loma population (9.3179°N, 82.1547°W, Fig. 1), which is 6500 m from Bastimentos West, are monomorphic. These frogs almost always lack spots on the dorsum but some individuals have small black spots. Background dorsal coloration is bright orange to reddish and ventral coloration is white, occasionally with some mottling of the predominant dorsal background color.

MALE COLORATION

The spectral reflectances of the dorsum and venter of each male were measured using a StellarNet EPP2000Cs UV-Vis spectrometer (StellarNet, Inc., Tampa, FL) and an R600–8 reflectance probe. The light source was a combination of an SL1 Tungsten Krypton lamp and a SL1-Blue UV-LED lamp (StellarNet, Inc., Tampa, FL). To avoid effects of lamp drift on reflectance measurements, white standard measurements were taken between each individual.

Dorsal and ventral reflectances were compared in terms of their brightness and color contrast estimates using a *Dendrobates*

pumilio visual model (following Maan and Cummings 2009). This model incorporates the spectral sensitivity measures of the three cone classes of *D. pumilio* (Siddiqi et al. 2004) and estimates the perceived brightness contrast (ΔL) or color contrast (ΔS) of a male's reflectance as viewed against a green (*Heliconia sp.*) leaf under forest illumination (measured in *D. pumilio* habitats in Bocas del Toro, Panama).

EXPERIMENTAL TREATMENTS AND SETUP

A series of three-way choice experiments were conducted to assess the preferences of female *D. pumilio* for differently colored males. In these experiments, females with three phenotype/population combinations (red and yellow from population A and red-orange from population B) were each presented with a set of three stimulus males with these same phenotype/population combinations. This allowed us to assess whether preferences for “own” versus “other” phenotypes differed between females from the polymorphic population (Bastimentos West) and the monomorphic population (La Loma).

The experimental setup was similar to Maan and Cummings (2008) but with modifications to accommodate a choice of three, instead of two focal males. Female chambers were

UV-transparent, clear acrylic boxes (40 × 20 × 10 cm). Male chambers were half-cylinders with a radius of 10 cm and a height of 20 cm. Visual barriers were placed between male chambers to prevent the stimulus males from viewing each other during the experiment. Trials took place in a dark room and 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 + CyanGel 4315) to mimic light conditions on the forest floor (Maan and Cummings 2008).

Male *D. pumilio* will occasionally call during preference trials (Maan and Cummings 2008, 2009). To avoid the potential effects of stimulus males' calls on female preferences, previous studies have either (1) presented females as stimuli instead of using male stimulus frogs (Summers et al. 1999; Reynolds and Fitzpatrick 2007) or (2) used males as stimuli and broadcast male calls from a speaker behind the male chambers to override any potential effects of the calls (Maan and Cummings 2008, 2009). Male *D. pumilio* are territorial (Bunnell 1973) and calling can be evoked by playing back recordings of other males' calls (Robakiewicz 1992; Paez et al. 1993). With this in mind, we conducted a pilot experiment (10 trials) using males as stimuli but without broadcasting male calls. None of the stimulus males called during these trials so we conducted the remainder of our choice trials without broadcasting male calls. Trials in which a stimulus male called were excluded from analyses.

EXPERIMENTAL PROCEDURE

We conducted a total of 134 female preference trials using 35 red females and 44 yellow females from population A, 54 females from population B, and 11 trios of stimulus males. Each female was tested once and male trios were used for a maximum of four trials per day.

Because frogs from population B are smaller than frogs from population A, trios of stimulus males were not matched for size and weight. Size matching would have required us to pair reproductively immature males from population A and reproductively mature males from population B. Because sexually mature males from these populations have dark throat patches and may exhibit different behaviors than immature males, we chose to present sexually mature but differently sized stimuli rather than equally sized stimuli at different stages of sexual development. Red and yellow stimulus males from population A however, were matched for size and weight within each stimulus trio.

Females acclimated to their chambers for at least 30 min and males for at least 15 min prior to experiments. During acclimation, males and females were prevented from interacting via a visual barrier. At the end of the acclimation period, the female was placed under a glass dome in the middle of her chamber and the visual barrier was removed, allowing her to observe the three males for a period of 2 min. The glass was then lifted and the trial

began when the female approached one of the males to within the "interaction zone," which was defined as a distance of two body-lengths (~4 cm, following Maan and Cummings 2008). Trials in which females failed to enter the interaction zone within 15 min were excluded from analysis. This 15-min threshold for interaction is intended to exclude females that are not sexually receptive (Maan and Cummings 2008).

After a female entered an interaction zone, we recorded the amount of time she spent in the interaction zone of each male and the number of times she approached each male's chamber within a span of 15 min. These two variables (interaction and approach) are thought to correlate with mating probability based on field observations (Maan and Cummings 2009). During the 15-min trial period, we also recorded whether any of the stimulus males called. To avoid potential side biases, this experimental procedure was repeated with the stimulus males in different positions. Immediately after the first 15-min trial, the visual barrier was inserted and the female was again placed under the glass dome while the positions of the three males were switched. The barrier was then removed and the female was again allowed to observe the males from under the glass for 2 min before the glass was removed and behavioral observations resumed. If the female did not enter an interaction zone within 15 min, the trial was terminated and the data were excluded from analysis. If the female entered an interaction zone, her behavior was again recorded for a period of 15 min. Only trials where the female entered an interaction zone within both 15 min halves of the trial and did not show a side bias (i.e., spend >80% of total interaction time in front of either the left, center, or right male chamber) were included in analyses.

DATA ANALYSIS

All statistical tests were carried out in SPSS 17.0 (SPSS, Inc., Chicago, IL). One-way analyses of variance (ANOVAs) and Tukey HSD tests were used to test for differences in coloration (reflectance) and size (SVL and weight) between stimulus males. These variables were normally distributed (all Shapiro-Wilk $P > 0.211$) and equal in variance (all Levene's $P > 0.107$). One- and two-way repeated measures ANOVAs were used to test for differences in square-root transformed female preference variables (interaction time and number of approaches) due to (1) the female's population of origin and (2) male phenotype. These variables were normally distributed after square-root transformation (all Shapiro-Wilk $P > 0.112$) and equal in variance (all Levene's $P > 0.088$).

Results

MALE PHENOTYPES

Although ventral reflectance measures did not differ significantly in terms of brightness (ΔL) and color contrast (ΔS) among the

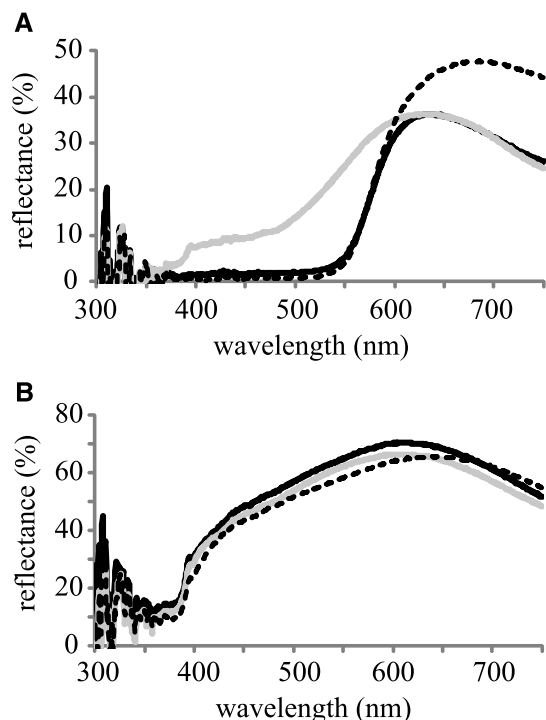


Figure 2. Average reflectances (A, dorsal and B, ventral) of males used in the experiments showing differences in dorsal, but not ventral reflectance among phenotypes: Bastimentos West, red (solid black line) and yellow (solid gray line), and La Loma (dashed black line).

three types of stimulus males (ΔL : ANOVA, $F_{2,27} = 0.677$, $P = 0.517$; ΔS : $F_{2,27} = 0.140$, $P = 0.870$; Fig. 2), dorsal reflectances did. From a *D. pumilio* point of view, yellow males from Bastimentos West have brighter dorsal reflectances (greater brightness contrast, ΔL) than red males from this population as well as males from La Loma (ΔL : ANOVA $F_{2,27} = 22.975$, $P < 0.001$, Tukey HSD $P < 0.05$), whereas the two other phenotypes (red males from Bastimentos West and La Loma males) did not differ in dorsal brightness contrast (Tukey HSD $P > 0.05$). Dorsal color contrast estimates (ΔS) were significantly different across all pairwise comparisons of the three phenotypes (ΔS : ANOVA, $F_{2,27} = 104.086$, $P < 0.001$).

La Loma stimulus males were smaller (means: SVL = 18.44 mm, weight = 0.53 g) than those from Bastimentos West (SVL: $F_{2,33} = 31.60$, $P < 0.0001$, Tukey HSD $P < 0.05$; weight: $F_{2,33} = 27.02$, $P < 0.0001$, Tukey HSD $P < 0.05$) but yellow (means: SVL = 19.89 mm, weight = 0.65 g) and red (means: SVL = 20.41, weight = 0.70 g) males from Bastimentos West did not differ in size from each other (SVL and weight: Tukey HSD $P > 0.05$).

FEMALE PREFERENCES

In total, we conducted 134 female preference trials. After removing trials where the female did not enter a male interaction zone

within 15 min (44 trials), trials where one or more stimulus males called (two trials) and trials where females showed a side bias (five trials), 83 (62%) trials were included in the analyses (26 red females and 30 yellow females from population A, 27 females from population B).

Red Bastimentos West females interacted with the three stimulus male types for unequal amounts of time (repeated measures ANOVA: $F_{2,50} = 3.19$, $P = 0.049$) but showed no difference in the number of times they approached each type of male (repeated measures ANOVA: $F_{2,50} = 1.64$, $P = 0.204$) (Fig. 3). These females spent the greatest amount of time interacting with males of their own phenotype and nearly equal amounts of time interacting with yellow males from their own population and males from La Loma, although none of these pairwise differences are significant (all Tukey HSD $P > 0.05$).

Yellow Bastimentos West females also interacted with the three stimulus male types for unequal amounts of time (repeated measures ANOVA: $F_{2,52} = 5.96$, $P = 0.005$). They spent significantly more time interacting with males of their own phenotype than they did with red males from their own population and red La Loma males (Tukey HSD $P < 0.05$). Yellow Bastimentos West females also approached their own phenotype significantly more often than they did either of the other two phenotypes (repeated measures ANOVA: $F_{2,52} = 6.17$, $P = 0.004$; Tukey HSD $P < 0.05$).

La Loma females interacted with the three stimulus male types for equal amounts of time (repeated measures ANOVA: $F_{2,58} = 1.34$, $P = 0.270$). However, they approached stimulus males with unequal frequencies (repeated measures ANOVA: $F_{2,58} = 5.23$, $P = 0.008$). They approached red Bastimentos West males more often than they did males from their own population or yellow Bastimentos West males (Tukey HSD $P < 0.05$).

REPRODUCTIVE CHARACTER DISPLACEMENT

To test whether females' preferences for males of their own phenotype were stronger in the polymorphic population (A, Bastimentos West) than in the monomorphic population (B, La Loma), we conducted two-way, repeated measures ANOVAs for both interaction time and number of approaches. In each of these tests the two factors were (1) the female's population of origin (A vs. B) and (2) potential mate phenotype (female's "own" vs. "other").

For interaction time, the population main effect was not significant ($F_{1,81} = 0.87$, $P = 0.354$) indicating that females from populations A and B did not differ in the amount of time they spent interacting with males. However, the phenotype main effect was significant ($F_{1,81} = 6.88$, $P < 0.010$), indicating that females spent more time interacting with males of their own phenotype than males of other phenotypes. The interaction term for this test was also significant ($F_{1,81} = 4.57$, $P = 0.036$). Bastimentos West

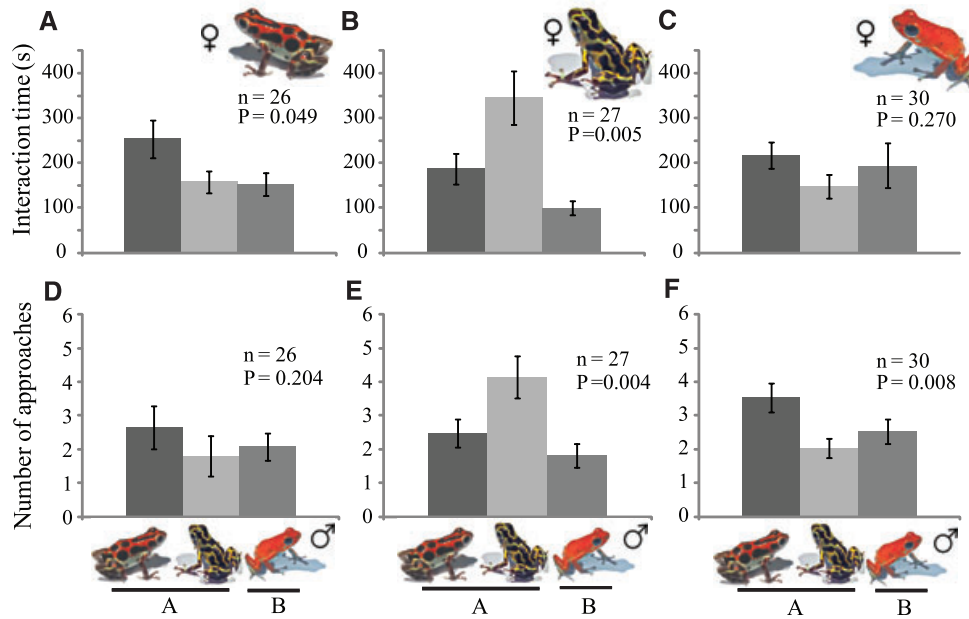


Figure 3. Results of mate choice experiment. Left (dark gray) bars on each plot correspond to red males and middle (light gray) bars to yellow males from Bastimentos West (A). Medium gray (right) bars correspond to males from La Loma (B). Top panel (A, B, and C) is female interaction time with each male phenotype: (A) red females and (B) yellow females from Bastimentos West, and (C) females from population La Loma. Bottom panel (D, E, and F) is number of approaches to each male phenotype: (D) red females and (E) yellow females from Bastimentos West, and (F) females from La Loma. Error bars are standard errors of the mean.

Females showed a stronger preference for interacting with males of their own phenotype than did La Loma females.

The two-way repeated measures ANOVA for number of approaches showed the same pattern. Again the population main effect was not significant ($F_{1,81} = 0.37$, $P = 0.545$), in contrast to the phenotype main effect ($F_{1,81} = 4.71$, $P = 0.033$) and the interaction term ($F_{1,81} = 4.79$, $P = 0.0316$). This indicates that females from the polymorphic Bastimentos West population showed a stronger preference for approaching males of their own phenotype than did females from the monomorphic La Loma population.

Discussion

The phenotypic variation exhibited within and among *D. pumilio* populations in the Bocas del Toro archipelago provides a unique opportunity to elucidate factors important to the generation and maintenance of biodiversity. Previous studies have demonstrated that female mate choice is influenced by interpopulation differences in coloration and brightness, suggesting that sexual selection is important in maintaining patterns of diversity among allopatric populations (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008, 2009). However, the evolutionary processes generating these differences remain largely unclear.

A recent study by Maan and Cummings (2009) suggests that female preferences in some populations may be exerting directional selection on male coloration. Specifically, they found evidence for female preference for brighter males in several *D. pumilio* populations, including Bastimentos West. In highly variable populations, female preferences for extreme phenotypes can lead to disruptive selection and the maintenance of polymorphism (Pfennig and Pfennig 2009). In *D. pumilio* populations where females prefer brighter-than-average males, disruptive selection might be expected to result in polymorphic male coloration and female preferences. For example, if females prefer males with extreme coloration (e.g., the brightest yellows and brightest reds in Bastimentos West), males with these extreme phenotypes may have a fitness advantage over more intermediate phenotypes. This process is expected to result in a pattern of intraspecific RCD, where females in polymorphic populations show greater mating discrimination than females in monomorphic populations. Our study, which compares female mate preferences in polymorphic and monomorphic populations of *D. pumilio* on Isla Bastimentos, shows just such a pattern of intraspecific RCD, suggesting that selection has contributed to the generation and maintenance of intraspecific diversity. If diversifying selection on male coloration and female preference presents a strong enough barrier to reproduction among color morphs, this process could eventually lead to speciation.

Based on the properties of their visual system (Siddiqi et al. 2004), our visual modeling estimates suggest that frogs from the polymorphic and monomorphic areas of Isla Bastimentos differ in dorsal coloration and brightness and these differences are predicted to be perceptible by the frogs themselves. Our mate choice experiments revealed that when given a choice between males with their own phenotype, males with a different but sympatric phenotype, and males with a different but allopatric phenotype, females from Isla Bastimentos prefer to approach and interact with males of their own phenotype over males with other phenotypes. However, this preference for “own” versus “other” male phenotypes was stronger in females from the polymorphic population (both red and yellow variants) than in females from the monomorphic population, consistent with a pattern of intraspecific RCD.

The preference for “own” phenotype we observed was apparently stronger than a preference for “brighter” phenotype. Maan and Cummings (2009) found that when given the choice between two males of the same phenotype, female *D. pumilio* from several populations (including Bastimentos West) show a preference for brighter males. A preference for brighter males cannot explain the differential female response by population in the current study. If “brightness” preferences were the main force influencing female responses in these populations, then red females from Bastimentos West should prefer the yellow males from their population over red males. Our results showed the opposite, resulting in a pattern of preference consistent with intraspecific RCD. Females from the polymorphic population paid more attention to phenotype-matching, overriding a general preference for brighter males.

Several processes could have caused the pattern of RCD we observed on Isla Bastimentos (reviewed in Noor 1999). These include (1) reinforcement due to natural selection (Brown and Wilson 1956), (2) reinforcement initiated by natural selection but strengthened by sexual selection (Lande 1981, 1982; Coyne and Orr 1989), (3) differential fusion of populations with differing degrees of mating discrimination (Noor 1999), (4) interference in mate recognition signals by variants that do not actually hybridize (noisy neighbors, Otte 1989, see also Howard 1993), and (5) ecological variables that incidentally lead to changes in mating signals and preferences. Existing data do not permit discrimination among these alternatives for *D. pumilio*, although they do suggest avenues for future research, as we will presently discuss.

REINFORCEMENT

Reinforcement is traditionally thought of as being driven by natural selection, which strengthens sexual isolation in response to reduced hybrid viability or fertility (Brown and Wilson 1956; Noor 1999). However, recent authors have suggested that many

other types of costs to hybridization, including ecological, behavioral, and genetic dysfunctions, could reduce the fitness of hybrid offspring, driving reinforcement (Lande 1982; Coyne and Orr 1989; Noor 1999; Ortiz-Barrientos et al. 2009). Failure of hybrid offspring to secure mates (due to maladaptive female preferences and/or male signals) could drive reinforcement just as easily as intrinsic postzygotic isolation because hybrids that are sterile are evolutionarily no different than hybrids that cannot mate (Noor 1999). Males that waste time and energy courting females who always reject them might also incur a greater predation risk. In this way, costs involved in simply trying to hybridize could drive the evolution of prezygotic isolation (Higgie et al. 2000; Ortiz-Barrientos et al. 2009).

Although mating has been observed between differently colored frogs in the polymorphic area of Isla Bastimentos, it is not known whether offspring resulting from such matings show reduced fitness. Offspring from experimental crosses between allopatric color morphs are viable (Summers et al. 2004), but whether the sympatric morphs from Isla Bastimentos produce viable offspring, and whether they are equally as viable as offspring from within-morph matings remains unclear. Nothing is known about the fertility of intermorph offspring or whether they incur fitness costs due to maladaptive male phenotypes or female mate preferences.

The offspring from experimental crosses between allopatric color morphs show phenotypes intermediate between those of their parents (Summers et al. 2004). If this is the case for offspring resulting from intermorph matings on Isla Bastimentos as well, such offspring could suffer reduced fitness due to (1) increased predation (intermediate phenotypes may not be recognized and avoided), (2) inappropriate male signals (females may reject intermediately colored males), or (3) inappropriate female mate preferences (if intermediately colored females prefer intermediately colored males, e.g., Melo et al. 2009). Studies addressing how genetic, ecological, and behavioral aspects of fitness differ for offspring from within- versus among-morph matings would go a long way toward clarifying reinforcement’s role in the rapid evolution of morphological diversity in Bocas del Toro’s *D. pumilio*.

DIFFERENTIAL FUSION

The idea of differential fusion posits that a pattern of RCD could result from differences in the strength of mating discrimination among populations or variants. If only color morphs with strong mating discrimination persist in sympatry whereas morphs with weak discrimination fuse, the polymorphic populations we observe in *D. pumilio* may be a nonrandom sample of all morphs that have come into contact with one another (reviewed in Noor 1999). This hypothesis predicts greater postzygotic isolation between sympatric color morphs than allopatric ones and that the levels of prezygotic isolation observed among sympatric morphs form

a subset of those observed among allopatric morphs. Although prezygotic isolation (in the form of female mate preferences) has now been compared among a number of allopatric color morphs (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008, 2009), this study represents the first look at prezygotic isolation among sympatric morphs. Studies of prezygotic isolation in other polymorphic areas of Bocas del Toro and comparisons of postzygotic isolation between sympatric and allopatric color morphs are needed to assess the likelihood that differential fusion has resulted in the observed pattern of RCD.

NOISY NEIGHBORS

A pattern of RCD can result from selection acting to reduce interference between the mate recognition of nonhybridizing species (noisy neighbors hypothesis, Otte 1989; Howard 1993; Noor 1999; Mullen and Andres 2007). Extending this concept to the intraspecific case, the pattern of RCD we observed in the polymorphic Bastimentos West population could have been driven by selection to reduce interference in mate recognition signals between morphs that never actually hybridize. However, as matings between different morphs have been observed in nature, this explanation seems unlikely to explain the pattern of RCD described herein. If future studies show that offspring from such matings are inviable and hybrids never occur in nature, this hypothesis should not be ruled out.

ECOLOGICAL VARIABLES

If an ecological variable allows the presence of one color morph and affects mating discrimination in the other, the pattern of RCD we observed could have resulted from plasticity or selection in response to this variable (Noor 1999). Similarly, ecological character displacement may have incidentally caused changes in mate-recognition signals that make sympatric color morphs less likely to mate with one another. Although it is difficult to envision an appropriate scenario for the first case, it is somewhat easier to imagine differences in the ecology of sympatric color morphs of *D. pumilio* incidentally leading to differences in mate-recognition signals. For example, such a pattern might result if color morphs have different niches, leading to differences in predation among morphs. Studies comparing the ecological niches of sympatric color morphs might shed light on the likelihood that this hypothesis explains the pattern of RCD described here.

CONCLUSION

The spectacular variation in color and pattern among populations of *D. pumilio* in the Bocas del Toro region of Panama has long fascinated biologists. Several recent studies have made great strides in understanding the geographic, behavioral, and ecological factors involved in shaping patterns diversity among populations (e.g., Saporito et al. 2007; Maan and Cummings 2008;

Wang and Shaffer 2008; Brown et al 2010; Wang and Summers 2010). However, much less attention has been paid to the factors that affect within-population diversity in this species. Maan and Cummings' (2009) recent findings of directional selection on male coloration, coupled with the pattern of intraspecific RCD described herein provide the first glimpses into how selection acting within *D. pumilio* populations could drive evolutionary changes in male coloration and female preference. Over time, these same intraspecific processes, operating independently in many, geographically isolated populations, may have given rise to the variation we see across the Bocas del Toro archipelago today.

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