



RESEARCH PAPER

Color Assortative Mating in a Mainland Population of the Poison Frog *Oophaga pumilio*

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Abstract

Assortative mating is a reproductive strategy used by a diversity of animals, in which individuals choose a mate that shares similar characteristics. This mating strategy has the potential to promote the evolution of various sexual signals and has been a proposed mechanism driving and maintaining color variation in the anuran family Dendrobatidae. Most studies have examined this reproductive strategy in the polytypic poison frog, *Oophaga pumilio*, in the Bocas del Toro archipelago in Panama. Little attention, however, has been given to ancestral populations across this species' mainland range, where dramatic color polytypism appears to lack. Additionally, most studies are exclusively experimental and investigate mate choice between allopatric populations, neglecting the behaviors of naturally occurring mates. This study observed natural mating pairs within a population of *O. pumilio* on mainland Costa Rica and tested the prediction that color phenotype of mating females and males would be correlated. Naturally occurring pairs were found to share similar coloration, suggesting that color assortative mating operates in nature, and in a mainland population. Our results indicate that coloration is an important trait in driving the natural mate choices of female *O. pumilio*, which provides valuable insight into realistic mate selection tactics of this dendrobatid frog.

Introduction

The selective advantages of finding a suitable mate have led to the evolution of diverse and elaborate traits (e.g., plumage coloration in birds, iridescence in guppies, and large body size in lizards; Hill 1990; Endler & Houde 1995; Censky 1997), all of which are difficult to explain by natural selection alone (Andersson 1994). In systems where female choice drives sexual selection, a diversity of traits can be found among males as a mechanism to attract females (Emlen & Oring 1977; Ryan et al. 1982). Female mate choice involves actively and non-randomly choosing partners based on male signals (e.g., coloration, vocalizations) that may directly or indirectly indicate mate quality (Trivers 1972; Andersson 1994). Assortative mating is expressed in numerous animals, whereby individuals (mainly females) non-randomly choose

mates that share similar characteristics (Karlin 1978; Jiang et al. 2013). Assortative mating can influence the genotypes and phenotypes of populations, lead to the maintenance of local adaptation, and aid in prezygotic isolation, which could result in speciation events (Crespi 1989). Although a variety of different characters are used in assortative mating (e.g., body size, age; Censky 1997; Ferrer & Penteriani 2003), color-based assortative mating is particularly common among polymorphic and polytypic species (e.g., Lake Victoria cichlids, poeciliid fishes, dendrobatid frogs; Seehausen & van Alphen 1998; Endler 1983; Maan & Cummings 2008).

Anurans exhibit a wide range of color and pattern polymorphisms (reviewed in Hoffman & Blouin 2000), and the dendrobatid poison frog *Oophaga pumilio* represents a particularly well-studied, polytypic species, especially across populations found on

different islands of the Bocas del Toro archipelago in northwestern Panama. Conspicuous coloration in *O. pumilio* appears to be under natural selection, as an aposematic signal, warning potential predators of their unpalatable nature (e.g., Saporito et al. 2007; Paluh et al. 2014). Recent investigations, however, have evaluated the role of coloration in sexual selection and female choice (Graham-Reynolds & Fitzpatrick 2007; Maan & Cummings 2008). Females are the choosy sex in *O. pumilio*, and this strategy may have evolved due to the extensive parental care exhibited by *O. pumilio*, which includes female egg transport and daily tadpole feeding (Haase & Pröhl 2002; Savage 2002). Such behaviors may increase energy investments by females and lead to a shorter life span (Trivers 1972; Siddiqi et al. 2004; Dugas et al. 2015). A series of laboratory-based, experimental, dichotomous preference studies with populations of *O. pumilio* in Bocas del Toro, Panama, have found that females generally prefer to associate with males from their native population and color morph (e.g., Summers et al. 1999; Graham-Reynolds & Fitzpatrick 2007; Maan & Cummings 2008). These studies provide evidence of color-based assortative association preference in *O. pumilio* and suggest that color assortment has contributed to the high color polytypisms observed in this island system (see Gehara et al. 2013 for review and alternative hypotheses). To date, however, most female choice experiments have been conducted with frogs from different populations that are isolated on islands separated by salt water, which prevents natural pairings (Gerhardt 1982; Dreher & Pröhl 2014). Furthermore, frogs from one polymorphic population on Isla Bastimentos appear to preferentially associate based on color in laboratory trials, but not in the wild (Richards-Zawacki et al. 2012). The discrepancies between laboratory trials and natural interactions may be a result of the important difference between a female's preference for a similarly colored mate and the actual choice she makes given certain trade-offs faced in the wild, such as mate sampling or foraging time. These choices may increase her predation risk or decrease her foraging time. Therefore, field studies that examine natural mating interactions within populations are necessary not only to further understand the mate choices of female *O. pumilio*, but also to understand whether phenotypic diversification and reproductive isolation are plausible in this system (Maan & Cummings 2008; Meuche et al. 2013).

While many studies on mate preference in *O. pumilio* occur between the polytypic populations in Bocas del Toro, the majority of this species geographic

range lies on the mainland in northwestern Panama, north along the Caribbean coast of Costa Rica and into southern Nicaragua (Savage 2002). Across their mainland range, these frogs are relatively monomorphic, exhibiting only slight deviations from a red dorsum and blue-legged frog. Only a few studies have examined female mate choice in this part of the species range and have found that females tend to associate with males based largely on call properties and proximity (i.e., distance from female), which may be independent of color phenotype (Meuche et al. 2013; Dreher & Pröhl 2014). However, the specific role of color in female mate choice within a single population of the ancestral lineages of *O. pumilio* is not fully understood and requires further study.

The goal of this study was to observe naturally interacting mating pairs of *O. pumilio* in a population from northeastern Costa Rica to determine whether assortative mating occurs. We predict positive color-based assortative mating will occur, which could clarify the mechanisms of mate decisions in *O. pumilio* and help to explain the variation and maintenance of coloration exhibited by this frog species.

Materials and Methods

This field-based study was conducted at the Organization for Tropical Studies, La Selva Biological Research Station in northeastern Costa Rica (10°26'N, 83°59'W) from Jun. 26, 2014, to Jul. 21, 2014.

Behavioral Observations

Observations of naturally occurring mating pairs of *O. pumilio* were conducted daily from 0600 to 1200 and 1500 to 1700, during the times in which male calling and male/female courtship are most common (Limerick 1980; Gardner & Graves 2005; Willink et al. 2014). Males were located by following the sounds of their advertisement calls. Once located, a 2 m area surrounding the calling male was searched for the presence of a female. The observer stayed at least 1 m away from the male while searching, to avoid interfering with natural behavior. If a female was present within 2 m of the male, behavioral observations of the pair immediately began and lasted for 15 min. The observer stood at least 2 m away from the pair to avoid disrupting natural interactions (e.g., Limerick 1980; Willink et al. 2014). For each trial, we recorded the total time a male spent calling, the total number of calling bouts (short periods of intense calling, followed by a short break), the direction a male was facing while calling (toward or away from the female),

distance and direction males moved between call bouts, and the distance and direction a female moved.

Interactions between males and females were categorized into three types of events. An interaction was considered a 'non-mating event' if a female moved farther than 2 m away from a male during the 15-min observation period and did not return to within 2 m of the male within 5 min. 'Non-mating events' were not included in the analysis due to the uncertainty of the female's receptiveness to mate. An interaction was considered 'female interest' if a female stayed within 1 m of a calling male and associated with a male throughout the 15-min observation period, by which then both the male and female were captured, and taken back to the laboratory for measurements. Finally, an interaction was considered 'high female interest' if a male turned and moved away from a female, and the female then followed the male for at least 3 s. This particular behavior was deemed the 'follow behavior', and when it was observed, regardless of the time in the observation period, the male and female pair were captured and taken back to the laboratory for measurements. Many times, a male would move away from the female and the female would not follow him, so he would return to her and continue to call directly at the female. It was only considered a 'high female interest' event when the female followed the male, because it was presumed that at this point, the female was following the male to an oviposition site (Limerick 1980). In addition, female *O. pumilio* who are in contact with a male on a given day are highly likely to ultimately select that male to mate with (Meuche et al. 2013). Therefore, it was presumed that if a female interacted with a male for 15 min, a mating event was likely to occur.

The distinction between 'female interest' and 'high female interest' events allowed for a less rigorous measure of mating success and a more certain measure of mating success, both of which were used in the analysis. A number of reasons justified the inclusion of 'female interest' events. First, during the course of this study, some interactions were observed for longer than 15 min ($N = 7$ pairs; observation time = 16–66 min). All of these interactions would have been considered 'female interest' events after the 15-min time period used in this study. In all seven interactions, however, the 'follow' behavior was observed after 15 min, and therefore, the interaction was categorized as a 'high female interest'. Further, courtship in this species can last anywhere between 15 and 120 min (Savage 2002), and the pairs in this study could have been found at any point during courtship. For these reasons, and the logistic and time

constraints of the study, the behaviors used to categorize each 'female interest' event were considered an appropriate measure of mating success. Discussed below are the results from both the 'high female interest' pairs and the combined 'female interest + high female interest' events.

Color Measurements

The hue, brightness, and chroma of all paired males and females were measured using an Ocean Optics (Dunedin, FL, USA) USB 4000 UV-VIS spectrophotometer, with a PX-2 pulsed xenon light source and a R400-7-SR reflectance probe with a 400 μm core diameter. Color measurements were taken with the spectrophotometer held 6 mm away and at a fixed angle of 90° perpendicular to the dorsum of the frogs. Each frog was measured in a laboratory within 5 h of capture. White standard measurements were taken between each individual frog using a Labsphere certified reflectance standard to accommodate for changing ambient light in the laboratory. Three random points along the dorsum of each individual frog were selected, and three spectrophotometric readings were measured at each of these locations (Fig. 1). The nine measurements and resulting reflectance curves were then averaged together to obtain an average dorsal coloration for each individual frog. Hue, brightness, and chroma were calculated using the Java-based

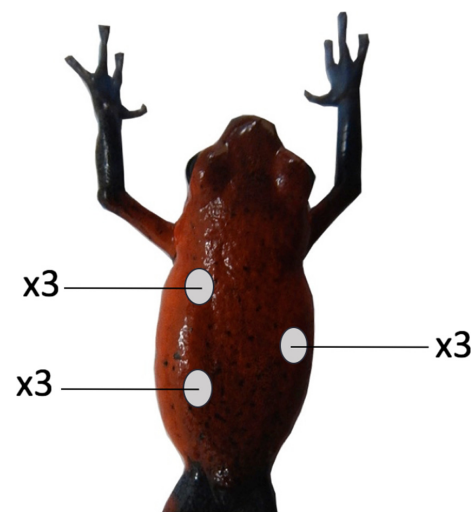


Fig. 1: Sampling schematic for measuring color of individual frogs using a spectrophotometer. Three points along the dorsum of the individual frog were selected and three measurements at each point were taken and then averaged together to obtain a final measure of color for the individual.

program CLR (version 1.05; Montgomerie 2008), which follow the equations detailed by Endler (1990).

Dorsal patterning was recorded by photographing the dorsum of each individual using a camera that was orientated on a tripod 15 cm above the frog. Each photograph was digitally analyzed using the computer program ImageJ (version 1.48; Rasband 2014). The percent of the dorsum covered with pattern was calculated by dividing the sum of the area for all blotches (i.e., pattern) by the total dorsal area of each frog. Snout-to-vent length (SVL) was measured to the nearest 0.01 mm using digital calipers, and mass was measured using a Pesola PPS200 digital pocket scale to the nearest 0.01 g.

Statistical Analysis

Canonical correlation analysis (CCA) was used to correlate hue, brightness, chroma, and pattern as a composite measure of phenotype between males and females. CCA assesses the relative importance of each color trait to the overall correlation and rank-orders the traits by importance. Analyses were conducted on 'high female interest' pairs and on 'high female interest + female interest' pairs. All analyses were conducted in R (R Core Team 2013), with an alpha of 0.05.

Results

A total of 64 mated pairs were observed and collected throughout the study, 35 'high female interest' pairs and 29 'female interest' pairs. The total reflectance curves for each pair can be seen in Figure S1 ('high female interest' pairs) and Figure S2 ('female interest' pairs). The coloration of the population of frogs at La Selva ranged in hue (0.22–0.70), brightness (0.07–0.48), chroma (11.83–95.10), and pattern (0%–18.47%) ($n = 478$). Female coloration ranged in hue (0.22–0.53), brightness (0.07–0.46), chroma (12.74–95.10), and pattern (0%–18.47%). Male coloration ranged in hue (0.28–0.70), brightness (0.08–0.48), chroma (11.83–91.93), and pattern (0.05%–11.10%).

There was no significant canonical correlation between male and female 'high female interest' or 'high female interest' + 'female interest' mated pairs when hue, brightness, chroma, pattern, mass, and SVL were included in the model ('high female interest': canonical correlation = 0.77, $F_{49,116.12} = 1.38$, $p = 0.08$; 'high female interest' + 'female interest': canonical correlation = 0.62, $F_{49,222.73} = 1.34$, $p = 0.08$; see Figures S3 and S4). Removing mass and SVL from the model, to focus solely on color-related

phenotypic attributes, yielded a strong and significant correlation in overall color and pattern phenotype between male and female 'high female interest' mating pairs (canonical correlation = 0.694, $F_{16,86.18} = 2.71$, $p = 0.001$; Fig. 2; see Figure S5), which is largely based on the contribution of chroma and brightness (standardized canonical correlates: $\text{chroma}_{\text{female}} = 0.888$, $\text{chroma}_{\text{male}} = 0.626$; $\text{brightness}_{\text{female}} = 0.810$, $\text{brightness}_{\text{male}} = 0.428$). There was also a strong and significant correlation in overall color and pattern phenotype between male and female 'high female interest + female interest' mating pairs (canonical correlation = 0.563, $F_{16,150.34} = 2.49$, $p = 0.002$), which is also largely based on the contribution of chroma and brightness (standardized canonical correlates: $\text{chroma}_{\text{female}} = 0.788$, $\text{chroma}_{\text{male}} = 0.815$; $\text{brightness}_{\text{female}} = 0.810$, $\text{brightness}_{\text{male}} = 0.710$).

Discussion

In natural mating interactions within a mainland population of *O. pumilio*, females were found to pair with males that look most similar to themselves with respect to color. These findings provide the first field-based evidence of assortative pairing within a population of *O. pumilio* based on dorsal coloration, suggesting that courtship pairing in this species is not random with respect to color, but instead involves

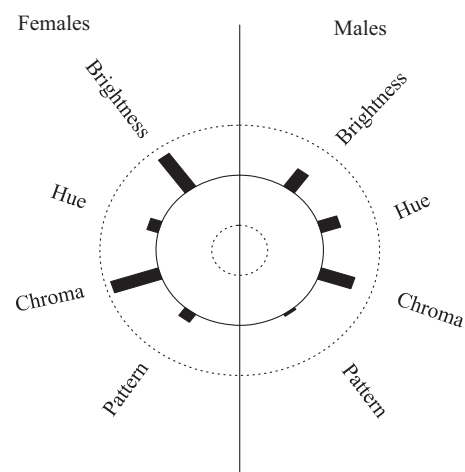


Fig. 2: Standardized canonical correlates of each phenotypic trait in the correlation between 'high female interest' mated pairs. The left side shows the contribution of each color trait to the overall correlation in females, while the right side shows the contribution of each color trait to the overall correlation in males. The length of each bar represents the relative contribution of the trait to the overall correlation for both males and females, with longer bars indicating a stronger correlation.

color-based selection by females. Assortative association preferences have been previously reported between different populations of *O. pumilio* in the Bocas del Toro archipelago using methods that have not included natural elements of mate choice (i.e., territories, mate searching, calling; e.g., Summers et al. 1999; Maan & Cummings 2008; Graham-Reynolds & Fitzpatrick 2007). The present study represents the first to find evidence of assortative pairing under completely natural conditions, taking into account the entire array of cost–benefit scenarios encountered by female *O. pumilio* within a mainland population.

Body size has been shown to be important for assortative mating in some systems (e.g., arthropods, fish, birds; Harari et al. 1999; Olafsdottir et al. 2006; Helfenstein et al. 2004); however, the results of the present study indicate that not only is size unrelated to color phenotype (Figures S3 and S4), but that body size (mass and SVL) is not a critical component in female choice. Pairs of male and female *O. pumilio* in the present study were most similar to each other in brightness and chroma, which have been shown in other studies to be tightly linked color components (Endler 1990; LeBas & Marshall 2000; Dugas & McGraw 2011), suggesting that brightness and chroma are the most important components driving mating assortment.

It is possible that separate components of color are under differential selective forces. Birds, which are presumed to be one of the main color-visioned predators of *O. pumilio* (Saporito et al. 2007; Paluh et al. 2014; Dreher et al. 2015), seem incapable of discriminating differences in the brightness among frogs within populations (Cummings & Crothers 2013). However, given that conspecifics (i.e., potential mating pairs) are capable of such discrimination (Dreher et al. 2015), it is possible that brightness and chroma are maintained mainly by sexual selection. In contrast, hue was found to be least important with respect to assortment, and therefore, hue may more strongly be influenced by natural selection, a conclusion consistent with a number of other studies (e.g., Noonan & Comeault 2009; Chouteau & Angers 2011; Amezcuita et al. 2013). Studies on other dendrobatids, such as *Oophaga histrionica*, *Ranitomeya imitator*, and *Dendrobates tinctorius*, have found novel color morphs to be selected by predators more often than local morphs, further suggesting that natural selection is acting on the hue of these frogs (Noonan & Comeault 2009; Chouteau & Angers 2011; Amezcuita et al. 2013). The findings of the present study, coupled with those of previous studies, suggest that brightness and

chroma are maintained mainly by sexual selection and hue is more strongly impacted by natural selection as an aposematic trait.

Male and female mated pairs of *O. pumilio* may have similar coloration for reasons aside from female preference, including microhabitat selection, which may influence predator detectability. Other polymorphic anurans have been shown to choose microhabitats that best match their phenotypes, presumably as a mechanism to decrease the probability of detection by predators (Morey 1990; Hoffman et al. 2006). It is possible that *O. pumilio* chooses a microhabitat to increase (or decrease) their conspicuousness as it relates to predator avoidance (Pröhl & Ostrowski 2011; Maan & Cummings 2012), and therefore, mate choice may be the result of spatial autocorrelation (Meuche et al. 2013). Additional studies will be necessary to better understand whether females actively choose males with similar coloration, or whether similarly colored pairs occur as a result of closeness and habitat choice.

Female *O. pumilio* may be utilizing multimodal signaling, whereby the females assess multiple sensory cues from males (i.e., color and calling) to select a mate. Previous studies have indicated the importance of male advertisement calls to female mate choice (Pröhl 2003; Meuche et al. 2013), and calls may be used as an initial attractant signal before a potentially more informative signal (i.e., coloration) is delivered to a female (Ord & Stamps 2008; Dreher & Pröhl 2014). Multimodal signaling has been observed in other taxa, such as Cyprinid fish, which use both visual and olfactory cues to choose a mate (Kodric-Brown & Strecker 2001), and some birds and other anurans which use both coloration and song to assess a mate (Taylor et al. 2007; Taff et al. 2012). Multimodal signaling may have evolved as a way to increase mate choice selection efficiency by offering a greater estimation of overall mate quality, and each signal may exploit different sensory inputs of an individual (see Candolin 2003). Across taxa, mate selection is influenced by an array of signals, and given the importance of color, auditory cues, and the complex courtship in *O. pumilio*, females are likely using an array of signals to choose a suitable mate.

Conclusions

The present study evaluated biologically relevant mate choices within a population of *O. pumilio* in a natural setting and found that color-based assortative pairing is occurring within this mainland population. This study, coupled with others, indicates that a large

suite of male characteristics and behaviors are responsible for female mating choices. This study provides valuable insight into realistic female mating decisions and offers insight into the plausibility of previous experimental mate choice studies in this system.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's website:

Figure S1. Total reflectance curves for ‘high female interest’ pairs. Red lines are females, and blue lines are males.

Figure S2. Total reflectance curves for ‘female interest’ pairs. Red lines are females, and blue lines are males.

Figure S3. Regression analyses showing the relationship between male mass and hue ($r^2 = 0.02$, $p = 0.43$), male mass and brightness ($r^2 = 0.03$, $p = 0.31$), male mass and chroma ($r^2 = 0.03$, $p = 0.32$), male SVL and hue ($r^2 = 0.04$, $p = 0.26$), male SVL and brightness ($r^2 = 0.01$, $p = 0.55$), and male SVL and chroma ($r^2 = 0.007$, $p = 0.62$).

Figure S4. Regression analyses showing the relationship between female mass and hue ($r^2 = 0.001$, $p = 0.83$), female mass and brightness ($r^2 = 0.009$, $p = 0.59$), female mass and chroma ($r^2 = 0.01$,

$p = 0.49$), female SVL and hue ($r^2 = 0.007$, $p = 0.63$), female SVL and brightness ($r^2 = 0.01$, $p = 0.56$), and female SVL and chroma ($r^2 = 0.01$, $p = 0.48$).

Figure S5. Regression analyses showing the relationships between male and female hue ($r^2 = 0.15$, $p = 0.02$), male and female brightness ($r^2 = 0.15$, $p = 0.02$), male and female chroma ($r^2 = 0.18$, $p = 0.006$), male and female pattern ($r^2 = 0.05$, $p = 0.09$), male and female mass ($r^2 = 0.002$, $p = 0.80$), and male and female SVL ($r^2 = 0.02$, $p = 0.47$).