



## Original Article

# Male–male contest limits the expression of assortative mate preferences in a polymorphic poison frog

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Codivergence of sexual traits and mate preferences can lead to assortative mating and subsequently reproductive isolation. However, mate choice rarely operates without intrasexual competition, and the effects of the latter on speciation are often overlooked. Maintaining trait polymorphisms despite gene flow and limiting assortative female preferences for less-competitive male phenotypes are two important roles that male–male competition may play in the speciation process. Both roles rely on the assumption that male–male competition limits the expression of divergent female preferences. We tested this assumption in the highly color-polymorphic strawberry poison frog (*Oophaga pumilio*). Females prefer males of the local color, suggesting that reproductive isolation may be evolving among color morphs. However, this inference does not account for male–male competition, which is also color-mediated. We housed females with two differently colored males, and compared reproductive patterns when the more attractive male was the territory holder *versus* when he was the nonterritorial male. Females mated primarily with the territory winner, regardless of coloration, suggesting that when a choice must be made between the two, male territoriality overrides female preferences for male coloration. Our results highlight the importance of considering the combined effects of mate choice and intrasexual competition in shaping phenotypic divergence and speciation.

**Key words:** assortative mating, female choice, male–male competition, *Oophaga pumilio*, reproductive isolation, sexual selection.

## INTRODUCTION

Understanding the mechanisms by which sexual selection drives speciation has been a growing focus in evolutionary biology (Ritchie 2007; Kraaijeveld et al. 2011; Servedio and Boughman 2017). Sexual selection can cause rapid codivergence of mating signals and mate preferences, potentially leading to reproductive isolation between divergent phenotypes (Lande 1981; Kirkpatrick 1982; West-Eberhard 1983). However, sexual selection encompasses both female mate choice and male–male competition, and the role of male–male competition has been largely ignored in speciation research (Qvarnström et al. 2012; Tinghitella et al. 2017; Lipshutz 2018). This is a major oversight because sexual signals often function both in female choice and male–male competition (Andersson 1994; Berglund et al. 1996; McCullough et al. 2016). Signals or weapons used in male–male contests can convey information on male quality, and drive the evolution of female preferences on these

male traits (e.g., skrraa calls in the Chlamydera bowerbirds; Borgia and Coleman 2000). Traits can also evolve first as courtship signals, and subsequently be co-opted to signal aggression or dominance in male–male contests (e.g., vertical bars in male swordtails; Morris et al. 2007). Because divergence of sexual signals can affect both female choice and male–male competition, without considering both processes, our understanding of speciation by sexual selection is incomplete at best.

Sexual selection arises due to asymmetries in mate limitation (i.e., a skewed operational sex ratio) and/or reproductive fitness gain per additional mating (i.e., the Bateman gradient) between the two sexes (Kokko et al. 2012). In most animal systems, females are choosy because they are able to choose from a large pool of potential mates and have less to gain from additional matings than males. This sexual selection paradigm underlies a mainstream view that female choice and male–male competition should be mutually reinforcing; that high-quality males are both competitively superior and preferred by females (Cox and Le Boeuf 1977; Berglund et al. 1996; Wong and Candolin 2005). While this is true in many systems, the pattern is far

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from universal (Qvarnström and Forsgren 1998; Wong and Candolin 2005; Hunt et al. 2009). The reproductive interests of males and females are often at odds, resulting in misaligned or even opposing inter- and intrasexual selection (Arnqvist and Rowe 1995; Moore and Moore 1999). Quantifying the interaction between female choice and male–male competition is especially crucial in speciation research because it dictates not only sexual signal evolution but also nonrandom mating patterns, and consequently, gene flow among the divergent phenotypes.

Recent research and syntheses have identified two potential roles of male–male competition in speciation by sexual selection. First, theoretical studies suggest that speciation via divergent female preference can only occur under a limited set of conditions, and these conditions are especially limited when there is gene flow between the incipient species. A key challenge to speciation by sexual selection is maintaining mating trait and preference variation during the speciation process (Arnegard and Kondrashov 2004; van Doorn et al. 2004). Recent studies suggest that male–male competition can maintain this variation if males bias their aggression toward phenotypically similar rivals (van Doorn et al. 2004; Mikami et al. 2004; Seehausen and Schluter 2004). However, whether this promotes speciation depends critically on the assumption that the advantage gained in male–male competition (e.g., holding a territory) translates to higher reproductive success despite assortative female preferences (Dijkstra et al. 2008). Second, divergent male types (or closely related species) are often asymmetric in competitive ability (Pryke and Griffith 2006; Sefc et al. 2015; Martin et al. 2017). Females may not be able to choose their preferred phenotype when nonpreferred males are better competitors (Reichard et al. 2005; Dijkstra et al. 2008), thus limiting mate preferences from translating to assortative mating. Such asymmetric male dominance could lead to competitive exclusion of the weaker phenotype or directional introgression of the stronger phenotype (Parker and Partridge 1998; Pearson and Rohwer 2000; Teeter et al. 2008; Sefc et al. 2015; While et al. 2015), breaking down the potential for sexual isolation due to divergent female preference. Testing the hypothesis that the outcome of male–male competition can limit or prevent the expression of divergent female preferences is critical to understanding the role of male–male competition in speciation.

The strawberry poison frog (*Oophaga pumilio*) exhibits extreme, heritable color polytypism in the Bocas del Toro region of Panama (Summers et al. 2003). Most described color variants occur among isolated island populations, but there are a few populations that show sympatric color polymorphism (summarized in Yang, Servedio, et al. 2019). Coloration in males and females is qualitatively similar in this species (Summers et al. 2003; Maan and Cummings 2009) and functions both as an aposematic signal (Saporito et al. 2007) and in intraspecific communication (Maan and Cummings 2008; Crothers and Cummings 2015; Yang et al. 2018). Females generally prefer males of the local color morph over an unfamiliar color morph (Summers et al. 1999; Reynolds and Fitzpatrick 2007; Maan and Cummings 2008; Richards-Zawacki and Cummings 2011), and this assortative preference pattern has been interpreted as evidence that sexual isolation is evolving among divergent color morphs. However, this inference does not account for male–male territorial competition, which is also mediated by color and has a substantial effect on male courtship success (Meuche and Pröhl 2011). Males respond more aggressively toward rivals of the local (familiar) color compared with an unfamiliar color in territorial contests (Yang et al. 2018; Yang, Servedio, et al. 2019). Males of conspicuous color morphs are also generally more aggressive than duller morphs (Rudh et al. 2013), suggesting the potential for asymmetry in competitive ability to evolve in association

with divergent coloration. Both patterns (aggression biases toward different-colored rivals and asymmetric aggressiveness among color morphs), as discussed above, have the potential to influence the evolution of reproductive isolation among *O. pumilio* color morphs.

Here, we conducted a breeding experiment with two territorial treatment groups in which male–male competition and female mate choice act either 1) in the *same* direction, or 2) in *opposing* directions. We allowed two size-matched males to compete for dominance, and subsequently introduced a female with a preference for the *winner's* or the *loser's* coloration. By comparing the mating patterns between the two territoriality treatments, we explicitly tested whether male–male territorial contests limited the expression of assortative color preferences in *O. pumilio* females. Testing this hypothesis is relevant to the role of male–male competition in both 1) maintaining sexual trait and preference polymorphism in the face of gene flow, and 2) limiting the expression of female assortative preference when nonpreferred phenotype is the superior competitor.

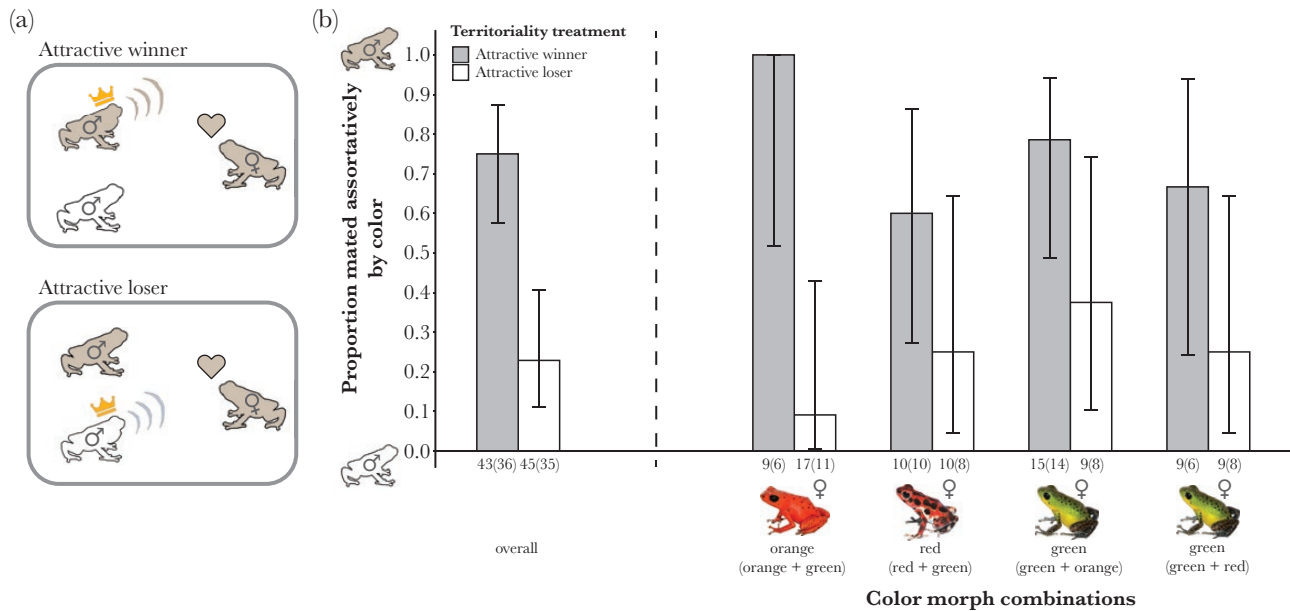
## METHODS

### Study species

*Oophaga pumilio* is a small terrestrial poison frog native and restricted to the Neotropics (Savage 1968). This frog is diurnal and has trichromatic color vision that enables it to distinguish variation in conspecific color signals (Siddiqi et al. 2004). Male *O. pumilio* defend territories year round through vocalization, visual displays, and physical combat (Bunnell 1973; Pröhl 1997; Pröhl and Berke 2001; Gardner and Graves 2005). Females have larger home ranges that overlap several males' territories, and they visit calling males within their home ranges when searching for potential mates (Pröhl and Berke 2001). Unlike most frogs, males of this species do not clasp females during mating; females can therefore terminate courtship at any time prior to mating (Yang, Blumenkamp, et al. 2019). When courtship is successful, females lay a clutch of ~5 eggs in the male's territory (Limerick 1980; Haase and Pröhl 2002). Although nonterritorial males often attempt to court females, there are no documented cases of successful mating resulting from this satellite strategy in the wild (Meuche and Pröhl 2011; Meuche et al. 2012). After oviposition, males guard and hydrate the terrestrial eggs. Upon hatching, females return to transport the tadpoles to individual nurseries (e.g., water-filled leaf axil) (Dugas 2018). Throughout larval development, tadpoles rely on the mother providing unfertilized eggs as their only food source (~45 days) (Weygoldt 1980; Dugas et al. 2016).

### Experiment design

Our breeding experiment had two territorial treatment groups in which male–male competition and female mate choice acted either 1) in the *same* direction, or 2) in *opposing* directions. Females were housed with two size-matched males, one of the same color morph as her own (her preferred color), and one of an unfamiliar color morph. We designed the trials so that the female was either the same color morph as the holder of the territory (terrarium) in which the three frogs resided (treatment AW, *Attractive Winner*) or the same color morph as the male who had lost the territorial contest (treatment AL, *Attractive Loser*; Figure 1A). We did not assay the females for color preference before introducing them into a trial. However, females of the three color morphs that we used (see below) have been shown to exhibit assortative color preferences in the wild (Maan and Cummings 2008; Richards-Zawacki



**Figure 1**

(A) Experimental design. In both treatments, the female was housed with a male of her own color morph and a male of a contrasting, unfamiliar color morph. In the *Attractive Winner* treatment, the territorial male was the same color morph (presumed to be her preferred morph) as the female; in the *Attractive Loser* treatment, the territorial male was the contrasting color morph (presumed to be less attractive). (B) The proportion of trials in which the female mated assortatively in the breeding experiment, overall (left of vertical dashed line) and broken down by color morph combination (right of vertical dashed line). Bars above  $y = 0.5$  indicate an assortative mating pattern, and bars below  $y = 0.5$  indicate disassortative mating. Error bars represent 95% binomial proportion confidence intervals. Images on the x axis show an exemplar of each color morph. Colors in parentheses indicate the color morphs of the two males with which the female was housed. Number of total trials and number of trials that successfully produced tadpoles within 200 days (in parenthesis) for each treatment and color morph combination are reported just below the x axis. Photographs by V. Prémel and S. A. Echeverri.

and Cummings 2011), and these preferences persist in pure-bred captive individuals in our breeding colony (preferences are shaped by maternal imprinting; Yang, Servedio, et al. 2019). We therefore assumed that the pure-bred females in our study displayed assortative preferences in our experiment (i.e., prefer males with their, and their mother's color compared with a contrasting, unfamiliar color). These trios were kept together until one pair produced tadpoles, which we then genotyped to reveal paternity as direct evidence of reproductive success. To achieve sufficient sample size in this experiment, individuals were reused across trials. However, a female was never asked to choose between males that she had previously been tested with. We record the IDs of the males and females and used appropriate statistical methods to account for potential individual variation in mating behaviors.

### Animals

We conducted our experiment using three color morphs in a breeding colony that was established from wild-caught individuals collected from three allopatric populations from Bocas del Toro, Panama: a bright orange-red morph from the southern tip of Isla Bastimentos (hereafter, *orange*), a red morph with coarse black patterning from the northwestern tip of Isla Bastimentos (hereafter, *red*), and a green morph with a bright yellow belly from Isla Popa (hereafter, *green*; exemplars of each morph are shown on the x axis of Figure 1B). Color is heritable in this species and all individuals used in the experiment were pure-bred (i.e., produced by individuals caught from the same wild population or their descendants). All animal enclosures were housed in the same walk-in environmental chamber (Darwin Chambers Company, St. Louis, MO) at the University of Pittsburgh. The chamber was maintained at 25 °C

and 70% relative humidity, under a 12L/12D light cycle. Frogs were fed with vitamin dusted fruit flies (*Drosophila melanogaster*). Other animal care and maintenance details were as described in a previous publication (Dugas and Richards-Zawacki 2015).

For both territoriality treatments, we used four different color morph combinations: 1) green female with green and red males, 2) green female with green and orange males, 3) orange female with orange and green males, and 4) red female with red and green males. We did not include the two combinations that asked the female to choose between orange and red males, because a previous study demonstrated that dorsal color hue (and not patterning) is the main target of female preference in *O. pumilio*, and that the red and orange morphs may not be different enough for the females to distinguish (Siddiqi et al. 2004; Maan and Cummings 2008). Color combinations were included as a categorical covariate of four levels in statistical analyses. Using multiple color morph combinations permitted us to ask whether the patterns we found were likely to be universal versus unique to certain phenotype combinations.

### Determining male territoriality

For each trial, we first introduced two differently colored, size-matched males into a 30 × 20 × 20 cm terrarium. Each terrarium floor was lined with moist sheet moss and each terrarium contained a live *Peperomia Scandens* vine for egg deposition, and four water-filled PVC tubes for tadpole rearing. These terraria were kept in the same environmental chamber and condition as the rest of the breeding colony and were misted with RO-filtered tap water several times a day. We observed the male pairs two to three times each week until the males established a stable hierarchy, operationally defined as the winner exhibiting behavioral dominance over

the loser for three consecutive observations. Dominant behaviors include calling, chasing, wrestling, and pinning, and submissive behaviors include escaping and freezing (Baugh and Forester 1994; Yang et al. 2018). During each hour-long observation, we observed up to 16 terraria via scan sampling, and recorded the dominant and subordinate males in each tank based on the tallied behaviors at the end of the observation. We marked a male as the territory holder (winner) when he was recorded as the dominant male in three consecutive observations.

### Mating trials

After the male pair had resolved their dominance hierarchy, we randomly introduced a female into the terrarium that was either 1) the same color morph as the winner (treatment AW), or 2) the same color morph as the loser (treatment AL). Females were assumed to display assortative preference (i.e., prefer males of the same color morph, see Experiment Design above). The tanks were censused every week for new tadpoles. Trials were terminated when the trio produced its first set of tadpoles, or when the trio failed to produce tadpoles in 200 days.

During the time that the trio were housed together, we continued to observe the behavior of the two males two to three times each week. Winner males were often observed courting females or acting aggressively toward the loser males (e.g., chasing, wrestling, and calling). We pooled these courtship and agonistic behaviors together because it is often difficult to tell whether the winner male was calling to the female or the loser male. Most loser males were socially inactive, but some displayed variable degrees of agonistic or courtship behaviors. To test whether the difference in loser behavior influenced the reproductive dynamics, we further categorized the losers as “submissive” or “aggressive” according to their behavior after the females were introduced to the terrarium. Loser males were classified as “submissive” when we did not observe any agonistic or courtship behavior during any of our observations, and classified as “aggressive” when agonistic and/or courtship behaviors were observed at least once.

We collected toe clips of adults and tail clips of tadpoles, extracted genomic DNA, and genotyped individuals at six polymorphic microsatellite loci (Hauswaldt et al. 2009) to determine paternity. With a known mother and only two candidate fathers in each trial, paternity could be assigned unambiguously by eye in all cases. We used tadpoles as our indicator of reproductive success because egg production is difficult to reliably monitor in the breeding colony (Dugas and Richards-Zawacki 2015). Tadpole production requires successful courtship, fertilization, egg development, male care (egg tending), and female care (tadpole transport), so our measurement likely underestimated the number of mating events. Previous studies in the same colony revealed that intra- and inter-morph breeding is similarly successful, suggesting no intrinsic reproductive barriers under captive conditions (Dugas and Richards-Zawacki 2015).

### Statistical analyses

To test the hypothesis that the outcome of male–male territorial contests limits a female from choosing her preferred color morph, we compared the mating pattern between the two territoriality treatments, using only the trials that produced tadpoles within 200 days. We tested the effect of territoriality treatment (AW/AL) on whether (yes/no) the female mated assortatively based on coloration (i.e., whether mate choice is in concordance

with presumed mate preference) using a binomial generalized linear mixed model (GLMM). In this model, we included color morph combination (four total combinations, see Methods) and loser behavior (submissive/aggressive) as covariates, and male and female IDs as random effects. We then tested whether bearing an attractive color trait can increase reproductive success on top of being territorial, with the prediction that the females are more likely to mate with a winner in the AW treatment compared with the AL treatment. We tested the effect of territoriality treatment (AW/AL) on whether (yes/no) the female chose to mate with the winner, including color morphs and the loser behavior as covariates, and male and female IDs as random effects. Because female preference for her own color is not absolute in *O. pumilio* (i.e., females do not choose assortatively 100% of the time), our analysis may have overestimated the effect of male territoriality on limiting assortative preference. Therefore, we conducted an additional analysis to compare the mating pattern found in this study with previously published estimates of female preference strength (see Supplementary Information and Figure S1 for details).

We then analyzed the factors that influenced the timing of reproduction (latency to produce tadpoles) using all data points (inclusive of trios that produced no tadpoles). We first compared the reproductive timing of the winners in the two territory treatments, modeling the effect of territoriality treatment (AW/AL) on the latency for winners to produce tadpoles using a Cox proportional hazards regression. We then compared the reproductive timing of the losers, similarly modeling the territoriality treatment's effect (AW/AL) on the latency for losers to produce tadpoles. Cox proportional hazards regressions test for effects of variables of interest on both the timing and the probability of occurrence of an event. Trials that terminated without the focal male successfully reproducing (i.e., trials terminated because the other male sired tadpole(s), or when the 200 d limit had been reached). Note that because there were trials in which neither the winner nor the loser produced any tadpoles in 200 days, the two Cox regression analyses were not identical. Color morph combination and loser behavior were included in both models as covariates.

All analyses were performed in R 3.6.2 (R Core Team 2019). We used the “glmmPQL” function in the *MASS* package (Venables and Ripley 2013) to fit the GLMMs. We used the “coxph” function in the *survival* package (Therneau and Grambsch 2000) to fit the Cox proportional hazards regressions. We tested the significance of the main effects (territoriality treatment and the covariates) using a likelihood ratio test with the “Anova” function in the *car* package (Fox and Weisberg 2018), which compares overall model fit with and without a particular effect. Post hoc Tukey pairwise comparisons of the significant main effects with more than two levels were made using the “glht” function in the *multcomp* package (Hothorn et al. 2008).

## RESULTS

We conducted a total of 88 trials, 71 of which successfully produced tadpoles within 200 days (mean  $\pm$  SD = 50  $\pm$  39 days, excluding trials that ended without tadpoles). Proportion of reproductive successes (number of trials resulting in tadpoles/total number of trials) in each treatment group were: *Attractive Winner*: 36/43 (84%), *Attractive Loser*: 35/45(78%); sample sizes for each color morph combination are listed below the  $x$  axis in Figure 1B. In 66% of the trials, we did not observe any agonistic or courtship behavior from

the loser male during any of our observations (“submissive losers”), while in the remaining 34% of the trials, the losers displayed some degree of agonistic and/or courtship behavior (“aggressive losers”). We were able to unambiguously assign parentage genetically for all tadpoles, and tadpoles in the same clutch (clutch size range: 1–7 tadpoles) were always sired by the same male. We used the 71 trials which successfully produced tadpoles in *Mating pattern* analyses, and all 88 trials in *Reproductive timing* analyses.

### Mating patterns

Territoriality treatment (AW/AL) had a significant effect on female mate choice (binomial GLMM,  $LR\chi^2 = 26.45$ ,  $df = 1$ ,  $P < 0.0001$ ): 75% of the females in the AW treatment mated assortatively by color, while only 23% of the females in the AL treatment did so (Figure 1B). Neither color morph combination nor loser behavior had a significant effect on female mate choice (color morph combination:  $LR\chi^2 = 1.62$ ,  $df = 3$ ,  $P = 0.654$ ; loser behavior:  $LR\chi^2 = 0.139$ ,  $df = 1$ ,  $P = 0.709$ ). Females were equally likely to mate with the winners of the same color morph (AW treatment) as with winners of an unfamiliar color morph (AL treatment; binomial GLMM,  $LR\chi^2 = 0.005$ ,  $df = 1$ ,  $P = 0.945$ ). Because this analysis used only trials that produced tadpole(s), the result also means that females were equally likely to mate with losers of either color. We did not detect a significant effect of color morph combination on the paternal identity of the tadpole(s) ( $LR\chi^2 = 6.57$ ,  $df = 3$ ,  $P = 0.087$ ); however, aggressive losers were more likely to sire tadpole(s) compared with submissive losers ( $LR\chi^2 = 7.21$ ,  $df = 1$ ,  $P = 0.007$ ).

### Reproductive timing

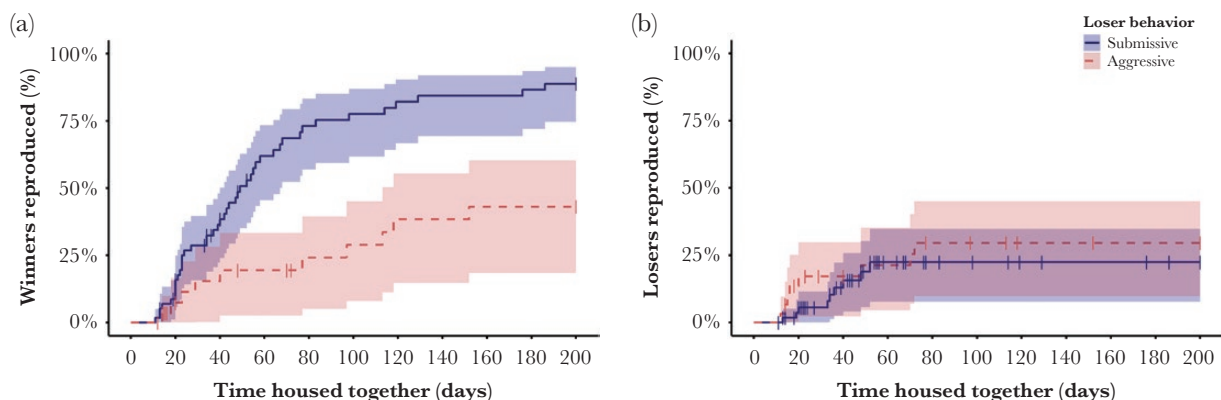
The reproductive timing of winners was not significantly different between the two territoriality treatments (Cox regression,  $LR\chi^2 = 0.057$ ,  $df = 1$ ,  $P = 0.811$ ). Color morph combination had no effect on the winner’s reproductive timing ( $LR\chi^2 = 1.71$ ,  $df = 3$ ,  $P = 0.635$ ); however, winners housed with an aggressive loser produced tadpoles significantly later compared with those housed with a submissive loser ( $LR\chi^2 = 16.56$ ,  $df = 1$ ,  $P < 0.0001$ , Figure 2A). In comparison, the reproductive timing of a loser was not predicted by territoriality treatment (Cox regression,  $LR\chi^2 = 0.064$ ,  $df = 1$ ,  $P = 0.801$ ) or by whether he behaved aggressively or submissively

( $LR\chi^2 = 1.71$ ,  $df = 3$ ,  $P = 0.635$ , Figure 2B), but was significantly different among color morph combinations ( $LR\chi^2 = 8.03$ ,  $df = 3$ ,  $P = 0.045$ ). However, none of the pairwise comparisons among the color morph combinations was significant in the Tukey post hoc tests (all  $P > 0.145$ ).

## DISCUSSION

We provide empirical evidence that supports the hypothesis that the outcome of male–male territorial contests can limit the expression of divergent color preference in the strawberry poison frog (*O. pumilio*), a species with highly divergent mating trait and mate preferences. We found that females mated primarily with the territorial winner, regardless of his coloration. Furthermore, the territorial losers of the female’s preferred color were no more reproductively successful than losers of an unfamiliar, less preferred color, suggesting that bearing an attractive color trait was not sufficient to rescue the reproductive success of a nonterritorial male. Building off the knowledge that both male competitive ability and aggression biases have diverged in concert with coloration (Rudh et al. 2013; Yang et al. 2018), our study demonstrated that these divergent male behaviors indeed interact with divergent female preferences to drive trait divergence and evolution of reproductive isolation in *O. pumilio*.

Although female color preferences had no effect on mating patterns in our experiment, we do not mean to suggest that color preferences have no effect on color evolution or reproductive patterns in nature. In the *Attractive Loser* treatment, the female could choose a mate bearing her preferred color or a mate with a territory but not both. In the wild, females may be able to sample among multiple male territories before mating, increasing the chance that she would encounter a male that is both territorial and bears her preferred color. In other words, color may not be important when a female is choosing between a territorial male and a nonterritorial male, but may become relevant when females are choosing between two territorial males. The number of potential mates a female can sample is often variable and restricted by social or ecological context, which dictates the sampling costs (Jennions and Petrie 1997; Rosenthal 2017). For example, population density is a strong determinant of how females sample potential mates in *O. pumilio* (as in many



**Figure 2**

Time-to-event curves showing the reproductive timing of territory winners (A) and losers (B) in trials with an aggressive (dashed line) or a submissive (solid line) loser. Shaded areas represent 95% confidence intervals, and vertical lines on the curve indicate censoring points for the survival analysis (i.e., trials terminated because the other male sired a tadpole(s), or the 200 d limit had been reached). The two territoriality treatments were pooled together because the treatment effect was not significant in the Cox regression analysis (see Results for details).

other animals; Kokko and Rankin 2006). Females in a low-density population mate with the closest calling male without comparing them to other potential mates (Meuche et al. 2013), but appear to be choosier in populations with higher densities (Gade et al. 2016; Yang, Blomenkamp, et al. 2019). The degree to which male dominance restricts the expression of female preference may therefore be variable among *O. pumilio* populations, potentially increasing as density decreases. However, even in populations where females sample multiple males, the outcome of male–male territorial competition can still determine the encounter rate of territorial males of her preferred versus less preferred color, indirectly restricting the expression of mate preferences.

In the wild, *O. pumilio* males that have lost a territorial contest may leave the location in search of another territory. However, it is also common for these nonterritorial males to stay and attempt to intercept and court females attracted by the winner's advertisement call (Meuche and Pröhl 2011). These satellite males court females by emitting quiet courtship calls to avoid detection by territorial males (Meuche and Pröhl 2011). We observed such behaviors in our experiment: 34% of the loser males in our trials attempted to court the females or exhibited some degree of agonistic behavior toward the territory winner. In the analysis using only the 71 trials that successfully produced tadpoles, females were more likely to mate with an aggressive loser compared with a submissive loser. However, trials with an aggressive loser were also less likely to produce tadpoles within 200 days compared with trials with a submissive loser (60% vs. 91%). When we considered these failed trials in the reproductive timing analyses, the reproductive success and timing of aggressive losers were not significantly different from submissive losers. On the other hand, the reproductive timing of the territorial winners was significantly delayed in the presence of an aggressive loser. This suggests that satellite males that are actively courting or fighting can inflict costs on the territorial owner even when territories do not change hands. This matches anecdotal observations in the wild, that females often lose interest in courtship when the territorial male engages in agonistic interaction with another male (Y. Yang, personal observation; H. Pröhl, personal communication).

A recent study revealed that both color-based female preference and male–male aggression biases in *O. pumilio* are formed through maternal imprinting instead of genetically inherited (Yang, Servedio, et al. 2019). Based on this finding, Yang et al. (2019) proposed a mechanism whereby this combination of learned behaviors may facilitate speciation by sexual selection. In this model, imprinted aggression biases generate negative frequency dependent selection, which can maintain a stable polymorphism and allow sexually imprinted female mate preferences to reduce gene flow in sympatry. Our findings in this study lend support to two important assumptions of the Yang et al. model (2019): that winning a territory confers major reproductive advantages to a male, and that aggression or challenges a territorial male receives decrease his reproductive success, even if he is able to maintain the territory.

The Yang et al. model (2019) incorporated individual male aggression biases toward rivals of different color morphs (Yang et al. 2018), but not asymmetric behavioral dominance among color morphs (Rudh et al. 2013). Females may not be able to choose males of their preferred color morph if competing morphs are superior in acquiring and defending territories. How this additional factor would impact the evolutionary trajectories described in the Yang et al. model (2019) is unclear. Intuitively, asymmetric dominance should disrupt reproductive isolation and make it more

difficult for a stable polymorphism to arise. However, the effect likely also depends on the degree of asymmetry in competitive ability and the relative strength of female choice and male–male competition. A more comprehensive analysis is required to test this verbal argument and explore the range of evolutionary outcomes that result from different scenarios.

Research on speciation by sexual selection has traditionally focused on divergent mate preferences, and studies elucidating the role of intrasexual competition are just starting to gain momentum (Tinghitella et al. 2017). Recent studies have proposed two main roles of male–male competition in speciation: 1) maintaining sexual trait and preference polymorphism in the face of gene flow, and 2) limiting assortative mating when males of the nonpreferred phenotype are superior competitors. Our study tested and confirmed a shared, critical assumption of both scenarios. Male–male competition does indeed limit the expression of divergent female mate preferences in *O. pumilio*, lending support to inferences made in previous studies. That sexual traits can mediate both female mate preference and male territorial competition (Andersson 1994; Berglund et al. 1996; McCullough et al. 2016), and that females pay attention to both male traits and territory status/quality (Jennions and Petrie 1997; Dijkstra et al. 2008) have been demonstrated across a range of animal taxa. The effect of male–male competition on speciation is likely to differ among taxa and be strongly influenced by certain aspects of the life history and ecology. One key factor is whether the preferred male trait and territory status/quality convey redundant versus nonoverlapping information about reproductive benefits to the assessing female. Knowing this would enable predictions of whether females are more likely to evaluate male trait and male territory independently, simultaneously, or weigh one over the other (Wong and Candolin 2005; van Doorn and Weissing 2004). In theory, male territory status/quality should have the largest effect on mating patterns in species where females gain a substantial reproductive benefit from a territorial male (e.g., parental care, reproductive resources). The degree to which females can resist forced or sneaked matings is also critical in predicting the importance of male–male competition in determining mating outcomes (Parker and Partridge 1998; Wong and Candolin 2005; Hunt et al. 2009). However, whether strong male–male competition facilitates or hinders speciation would also be influenced by the whether divergent male behavior takes the form of asymmetric behavioral dominance (likely hindrance), aggression biases (likely facilitation), or both (more complicated). Exploring whether the degree to which male territorial competition limits divergent female preference varies among species, and whether this variation correlates with these lineages' progression toward full reproductive isolation would be an exciting avenue for future research.

## SUPPLEMENTAL MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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