



# Prior residence effect determines success of male–male territorial competition in a color polymorphic poison frog

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## Abstract

Male–male competition shapes resource distributions and reproductive success among individuals, and can drive trait evolution when phenotypes differ in competitive abilities and/or strategies. Divergence of populations, regardless of the cause, is often accompanied by divergence in male competitive ability, and such asymmetries can play an important role in mediating the interactions and evolutionary trajectory of the nascent lineages. Here, we designed a field experiment to examine the importance of color, a divergent trait, in determining territorial contest outcomes in the poison frog *Oophaga pumilio*. Males of different *O. pumilio* color morphs differ in aggression level, suggesting a potential dominance hierarchy between these divergent phenotypes. In a contact zone between red and blue-color morphs, we first removed territorial males from their calling sites, and examined whether certain color morph(s) were better at establishing in these now-vacant territories. We then staged a territorial contest by simultaneously releasing the original and the new occupant to their point of capture. Surprisingly, we found no significant effect of color on acquiring territories or winning staged contests. However, the original occupants won against the new occupant in 84% of the staged contests, revealing a strong prior residence effect. This suggests that asymmetries that stem from prior residency override coloration in predicting contest outcomes of male–male territorial contests in wild *O. pumilio*. Thus, contradicting our hypothesis, male–male territorial competition alone seems unlikely to exert selection on coloration in this contact zone.

## KEYWORDS

color polymorphism, male–male competition, *Oophaga pumilio*, prior residence effect, territoriality, uncorrelated asymmetries

## 1 | INTRODUCTION

Animals often compete directly with each other for limited resources; in particular, males of many species engage in competitive contests for reproductive resources or access to mates (Andersson, 1994). Morphological traits that translate directly to fighting ability (i.e., resource-holding potential, or RHP; Parker, 1974) are often reliable predictors of contest outcomes. For example, animals with larger body or weapon size often dominate those with smaller body/weapon size

in a contest (McCullough et al., 2016; Prenter et al., 2008; Reichert & Gerhardt, 2011). However, because escalated physical combats are costly, many species evolve conspicuous signals that better communicate an individual's RHP. These signals aid the contestants in assessing each other, allowing them to settle conflicts without the unnecessary costs of escalated fights (Logue et al., 2010; Maynard Smith & Harper, 2003). Plumage coloration in birds (Keyser, 2000; Pärt & Qvarnström, 1997) and dominant call frequency in frogs (Arak, 1983; Bee et al., 1999) are both classic examples of such

honest signals (Zahavi, 1977). Variation in intrasexual competitive ability could also arise as a by-product via mechanisms initially unrelated to sexual selection. For example, darker body coloration has evolved repeatedly under selection for optimal thermoregulation (Trullas et al., 2007) or camouflage (Majerus & Mundy, 2003; Rosenblum, 2006). However, because many genes associated with melanin production also co-regulate hormone profiles and immunocompetence, darker morphs are often more aggressive and socially dominant over lighter morphs (reviewed in Roulin, 2016).

Regardless of the mechanism that generates the variation, divergence of populations is often accompanied by divergence in male competitive ability. Among closely related bird species, most interspecific aggression interactions are asymmetric (Freshwater et al., 2014; Martin et al., 2017). Similar aggression hierarchies are also found among divergent phenotypes of the same species (e.g., cichlids: Sefc et al., 2015; lizards: While et al., 2015; sticklebacks: Tinghitella et al. 2018). When these divergent phenotypes come into contact, competitive asymmetries can play an important role in mediating the interactions and evolutionary trajectory of the two lineages (Qvarnström et al., 2012; Tinghitella, et al., 2017). The stronger phenotype may exclude the weaker phenotype from reproductive resources, leading to asymmetrical introgression, hybrid zone movement, or even local extinction of the weaker lineage (Pearson & Rohwer, 2000; McDonald et al., 2001; Shurtliff et al., 2013; While et al., 2015). Quantifying competitive asymmetry among divergent phenotypes is therefore a critical component in understanding the processes of speciation and diversification.

The strawberry poison frog (*Oophaga pumilio*) exhibits extreme, heritable warning color polytypism in the Bocas del Toro region of Panama (Summers et al., 2003), and has become a popular system for research on population divergence in the past decade. The divergent trait, coloration, is known to mediate both female choice and male-male competition. Females, in general, prefer males of the same color morph over males of novel color morphs (Maan & Cummings, 2008; Reynolds & Fitzpatrick, 2007; Richards-Zawacki & Cummings, 2011; Summers et al., 1999; but see Yang et al., 2016), and this assortative preference pattern has been interpreted as an evidence that sexual isolation is evolving among divergent color morphs. The role of color-mediated male-male competition, on the other hand, is less well understood. Among allopatric color morphs, males differ in aggression level, with conspicuous color morphs generally more aggressive compared to duller morphs (Galeano & Harms, 2016; Rudh et al., 2013). This suggests a potential dominance hierarchy between the divergent phenotypes. Males also alter their aggressiveness when faced with rivals of different color morphs (Yang et al., 2018). However, while aggressiveness and social dominance are often correlated (Wilson et al., 2013), results from standard aggression assays do not always predict contest outcomes (Briffa et al., 2015; Earley et al., 1999). Testing whether variation in aggressiveness among allopatric color morphs translates to differences in competitive ability and contest outcome is critical to evaluating the role of asymmetric male aggression in trait evolution and population divergence in *O. pumilio*.

In this study, we took advantage of a phenotypic contact zone between red- and blue-color morphs of *O. pumilio*, and conducted

a field experiment to test whether males of different color morphs differ in their competitive ability in territorial contests in the wild. We first removed territorial males from their calling sites and examined whether coloration predicted which male would establish in these now-vacant territories. We then staged a territorial contest by simultaneously releasing the original territory holder and the new occupant at their point of capture, testing whether male coloration influences a male's ability to win a territorial dispute.

## 2 | METHODS

### 2.1 | Study species

*Oophaga pumilio* is a small terrestrial poison frog found in the Neotropics (Savage, 1968). Male *O. pumilio* defend territories year-round through vocalization, visual displays, and physical combat (Bunnell, 1973; Gardner & Graves, 2005; Pröhl, 1997; Pröhl & Berke, 2001). Females have larger home ranges that overlap several males' territories, and they visit calling males within their home ranges in search of potential mates (Pröhl & Berke, 2001). When courtship is successful, the female lays a clutch of ~5 eggs in the male's territory (Haase & Pröhl, 2002; Limerick, 1980). Females are unlikely to mate with a male without a territory (Meuche et al., 2012; Meuche & Pröhl, 2011), but there is no evidence that these territories provide reproductive resources to females (Dugas, 2018; Pröhl & Berke, 2001). After oviposition, males tend the eggs, and upon hatching, females return to transport the tadpoles to water-filled leaf axils (Killius & Dugas, 2014). Throughout larval development, the mother provides her tadpoles with her own unfertilized eggs as their only food source (~45 days, Dugas et al., 2016; Weygoldt, 1980).

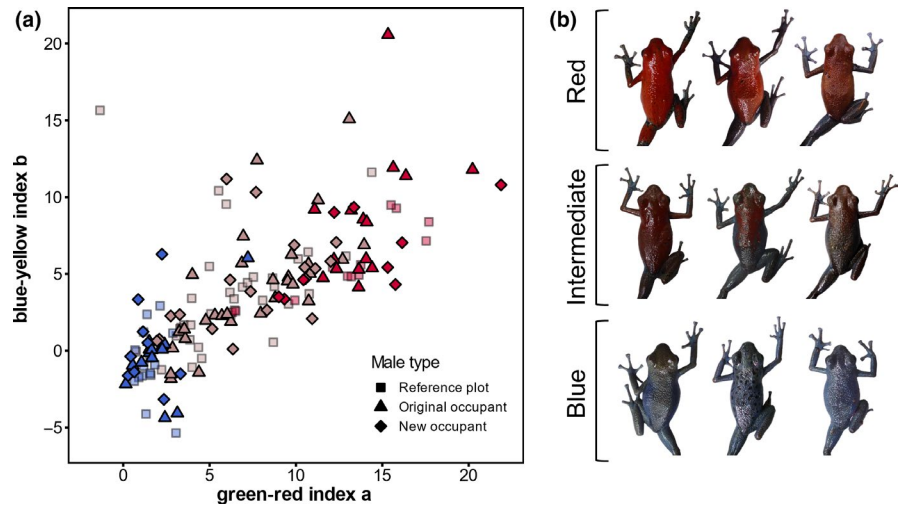
### 2.2 | Study population

We carried out our experiment in January–March, 2017, in a polymorphic contact zone between two interbreeding color morphs (red and blue) in the Bocas del Toro region of Panamá. Red, blue, and phenotypically intermediate frogs can be found in varying frequencies in this contact zone (Yang et al., 2016, 2018, 2019; Figure 1). We established a 20 × 20 m reference quadrat at our focal study site (a high-density, color polymorphic population) to estimate the color morph frequencies in the population. We exhaustively searched for and captured individuals in the quadrat (144 individuals total), measured their snout-vent lengths (SVLs), and took digital photographs for color analyses (see below).

### 2.3 | Experimental design

We designed a field experiment to explore whether there are any competitive asymmetries among these sympatric color morphs. We first removed the existing territorial males, and tested whether

**FIGURE 1** (a) By-eye color categories (color morphs) plotted in a quantitative color space based on photo-derived, hue-related color scores *a* (red-green) and *b* (blue-yellow). The three colors of the dots (roughly from left to right) represent by-eye categorization as blue, intermediate, and red, respectively. (b) Exemplars of color variation at the study site



non-territorial males of different color morphs differ in their ability to occupy these now-vacant territories (i.e., *establishment stage*; Figure 2 step 3). We then staged a territorial contest by simultaneously releasing the original and the new occupant to their point of capture, testing whether coloration influences the likelihood of winning a contest (i.e., *contest stage*; Figure 2 Step 5). To account for effects of body size and prior residency (i.e., original vs. new occupant), we also recorded these parameters and included them in our analyses.

## 2.4 | Experimental protocol

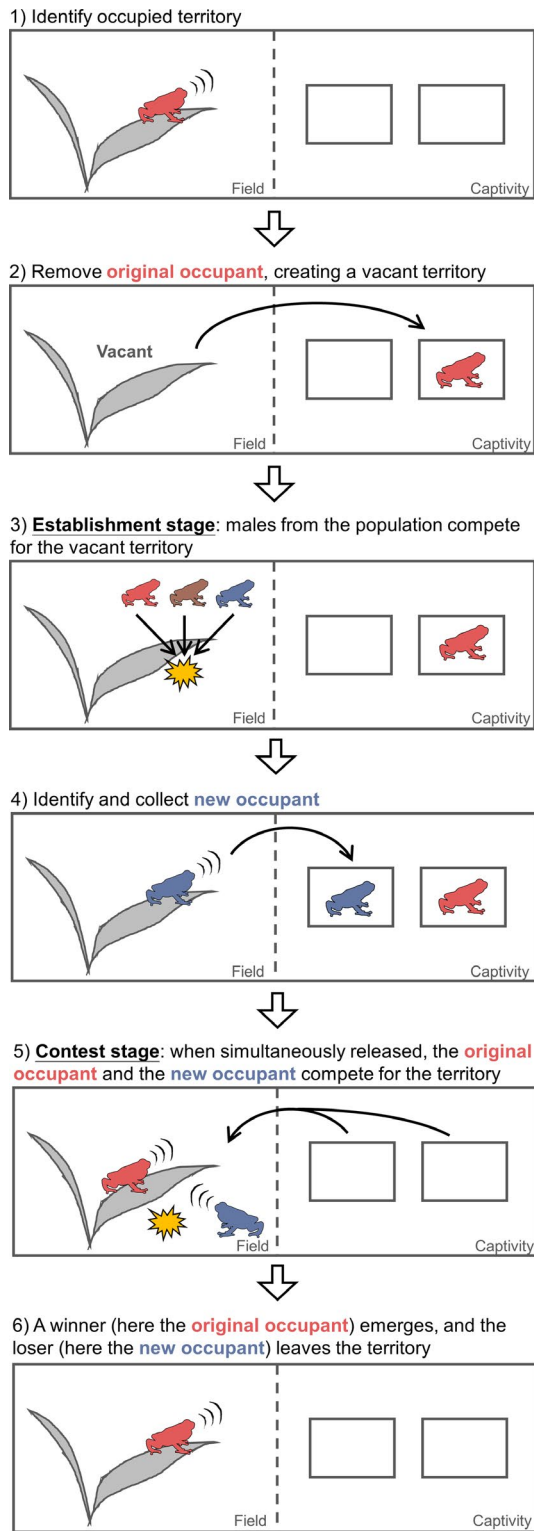
We first located territorial males by their advertisement calls, and used call playbacks to confirm territoriality (territorial males approach and call at playbacks, Yang et al., 2018; Figure 2 step 1). We then hand-captured the males and marked the locations with flagging tape. These males (the “original occupants”) were then transported to, and temporarily kept in captivity at the Smithsonian Tropical Research Institute’s Bocas del Toro field station (for frog housing and feeding protocols, see Yang et al., 2016; Figure 2 step 2). Because of the high population density and consequently high competition in this population, a new male (the “new occupant”) was often found occupying the vacant territory soon after the original occupant’s removal (mean  $\pm$  SE for re-occupancy of a site =  $11.7 \pm 9.5$  days). In this *establishment stage* (Figure 2 Step 3), we surveyed the flagged locations every 2–4 days for new calling males, and similarly used call playbacks to confirm territoriality. The territorial males were removed at different time points throughout the field season, and their territorial locations were continuously checked for at least 3 weeks and up to 5 weeks post-removal. When a new occupant was found, he was captured, brought back to the field station, and temporarily kept in captivity (Figure 2 step 4). All individuals captured (both the original and the new occupant) were given unique toe clips for identification (Funk et al., 2005). We measured SVL to the nearest 0.01 mm using a dial caliper, and categorized coloration by eye as red, blue, or intermediate. We also took digital

photographs (Panasonic DMC-TS5) of the dorsum against an 18% gray standard (DGK Color Tools).

During the *contest stage* (Figure 2 Step 5), we simultaneously released the original (the removed male) and the new occupant (the newly established male) to their point of capture, staging a contest between the two males. Because *O. pumilio* have good spatial memory and homing behavior (Mcvey et al., 1981; Nowakowski et al., 2013), both males likely recognized the release location as their territory. We observed both escalated physical combat and non-escalated contest resolution between the two individuals we released on several occasions. However, because resolving a territorial dispute is not always immediate and can take up to several days (Y. Yang *personal observation*), we did not record all behavioral interactions between the competing territorial males. Instead, we returned to survey the marked the males’ locations regularly (every 2–4 days) and used call playbacks to identify the contest winner (i.e., the territorial male at time of survey; Figure 2 Step 6).

## 2.5 | Color quantification

Because phenotypic intermediates between the red morph and the blue morph display continuous color variation (Figure 1b), we used both by-eye color categorization and objective quantitative color scores generated from digital photographs for our analyses (Dugas et al., 2015; Yang et al., 2018). To quantify color in a continuous way, we sampled red (R), green (G), and blue (B) values across five  $20 \times 20$  pixel areas on the frog’s dorsum using the software ImageJ 1.48v (Schneider et al., 2012). We calibrated the RGB values against the 18% gray standard in the same photograph (sampled similarly from a  $20 \times 20$  pixel area), then transformed the standardized RGB values to CIE (International Commission on Illumination) 1976  $L^*a^*b$  color space (CIELAB), generating three indexes: *L*, *a*, and *b*, corresponding to brightness, the green-to-red axis, and the blue-to-yellow axis, respectively. We applied this transformation because the RGB color space is not uniform, and differences in RGB values do not translate to equal differences in color perception (Stevens & Cuthill, 2005).



**FIGURE 2** Diagram showing the experimental procedure. Territoriality at steps 1, 4, and 6 was confirmed using call playbacks to elicit aggressive responses. Note that the color morphs of the males in the diagram are just an example; color combinations of the male pairs in the experiment emerged on their own in the field (i.e., we could not control what color morph would win in the establishment stage)

The CIELAB color space is designed to approximate uniform perception in human and not animal visual systems; however, CIELAB indexes are more biologically relevant than raw RGB values for frog color variation as perceived by the trichromatic *O. pumilio* (Siddiqi et al., 2004; Stevens & Cuthill, 2005). Correlation of photo-based CIELAB color scores and by-eye categorization were examined using ANOVAs testing for differences the *L*, *a*, and *b* indexes among the red, blue, and intermediate individuals.

## 2.6 | Statistical analyses

We first tested the prediction that the color morphs differ in their ability to establish in a vacant territory (*establishment stage*, Figure 2 Step 3) by comparing the color morph frequency of the new occupants with the population frequency using a two-sample chi-square test. Assuming equal accessibility of all frogs to the vacant territories, a more competitive color morph will have a higher frequency among the new occupants than its frequency in the population at large. We then tested the same hypothesis replacing the by-eye colors with the photo-based CIELAB color scores: We compared *L*, *a*, and *b* scores between the new occupants and reference plot males using three separate two-sample *t* tests (transformed to meet normality assumptions when appropriate). To test whether larger frogs are more likely to win a contest, we also compared body size (SVL) between the new occupants and reference plot males using a two-sample *t* test.

In these analyses, we compared males that claimed the vacant territories with that of all males in the reference plot, assuming that the latter represented the pool that non-territorial males came from. However, the presence of the territorial males alongside non-territorial ones in our reference plot could decrease our power to detect differences in competitive ability among the color morphs. For example, if red males were better territory holders and overrepresented among the territorial males (and underrepresented among the non-territorial males), using the color morph frequency of the reference plot (which includes both territorial and non-territorial males) and not solely the non-territorial males would make detecting higher red frequency among the new occupants more difficult. We unfortunately did not distinguish territorial male from non-territorial ones during our reference plot survey. We therefore conducted an additional analysis: We assumed that 25% of the males are territorial (likely an overestimate in this population, *unpublished data*), and that the color morph frequency of the new occupants was representative of the frequency of the territorial males in the population. We estimated the number of non-territorial males of each color morph in the reference plot by removing 25% of the males (chosen to match the color morph frequency of the new occupants) in this study. This would result in an adjusted reference frequency (calculated from the remaining 75% of the males) that presumably better represented the color frequency of the non-territorial males in the population. We then re-ran the chi-square test, comparing the color morph frequency of the new occupants to this

adjusted reference frequency. We were not able to do the same with the analyses on the photo-based CIELAB color scores or body size (SVL) because of the continuous nature of these variables.

We then examined whether the color morph of a male influenced the likelihood of winning the staged contest (*contest stage*; Figure 2 Step 5). Because of the limitation of small and unbalanced sample sizes in the contest stage, we only used photo-based, continuous color scores in these analyses. Using a generalized linear model (GLM) with binomial error structure, we tested the effects of *L* index difference, *a* index difference, *b* index difference on the staged contest outcome (original occupant won vs. new occupant won). We included SVL (body size) difference between the original and new occupant (original - new) as a covariate to account for potential effects of body size. To test the potential effect of residency status (original vs. new), we examined the effect of the GLM intercept. If dropping the intercept has no effect on model performance (i.e., winning likelihood = inverse logit of 0 = 0.5) that suggests that the original occupant and the new occupant are equally likely to win the contest. If, on the other hand, dropping the intercept has a significant effect, it suggests that the original occupant is significantly more likely to win (positive parameter estimate) or to lose (negative parameter estimate) the contest. We also performed a post hoc test to demonstrate the effect of residency status: We ran an exact binomial test on contest outcome, with the null expectation of the original occupant winning half of the contests (expected frequency = 0.5).

All analyses were performed in R 3.5.1 (R Core Team 2019). We used the “lm,” “chisq.test” function, “t.test” function, “glm” function, and “binom.test” function in the stats package (R Core Team 2019) to perform the ANOVA, two-sample chi-square test, two-sample *t* tests, GLM, and exact binomial test, respectively. We tested the significance of the main effects in the GLM using a likelihood ratio test with the “ANOVA” function in the car package (Fox & Weisberg, 2018), which compares overall model fit with and without a particular effect.

### 3 | RESULTS

We removed a total of 59 calling males from their territories, and successfully identified a new occupant claiming these vacant territories in 40 of the 59 vacant sites within  $11.7 \pm 9.5$  days (range 2–35 days). Because several of the removed males were used in a separate study, we did not stage a territorial contest between all 40 pairs of males. We released 23 original-new occupant pairs to their shared point of capture for staged contests. The original occupants were kept in captivity for  $13.6 \pm 9.4$  days (range 5–38 days), while the new occupants were kept for  $2.8 \pm 2.8$  days (range 1–11 days). Out of the 23 released pairs, we successfully identified a winner in 19 pairs. In the remaining four pairs, we found a third, unmarked individual occupying the territory in three, and did not find any males by the end of the experiment in one. We used all 40 new occupants for analyses in the establishment stage, and the 19 pairs in which we identified a marked winner for analyses in the contest stage.

From the 20 m × 20 m reference quadrat, we caught a total of 144 frogs (63 females, 61 males, and 21 juveniles). Color morph frequencies of females were 8% red, 25% blue, and 67% intermediate; color morph frequencies of all males were 20% red, 25% blue, and 55% intermediate.

#### 3.1 | Color quantification

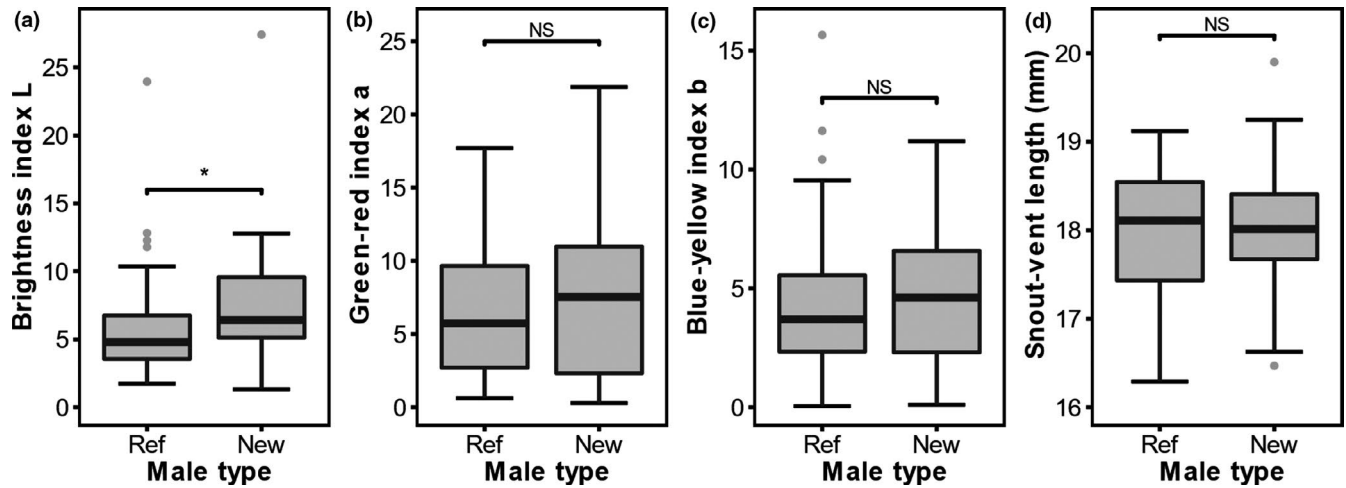
Photo-based CIELAB color scores correspond well to by-eye categorization (Figure 1a): Red, intermediate, and blue morphs differ significantly in both the green-red index *a* (ANOVA,  $F_{2,162} = 134.23$ ,  $p < .001$ ) and the blue-yellow index *b* (ANOVA,  $F_{2,162} = 48.73$ ,  $p < .001$ ), but not in the brightness index *L* (ANOVA,  $F_{2,162} = 1.21$ ,  $p = .301$ ). The *a* index was highest in red individuals, lower in intermediate individuals, and lowest in blue individuals (Tukey post hoc tests, all pairwise comparisons  $p < .001$ ). The *b* index was highest in red individuals, lower in intermediate individuals, and lowest in blue individuals (Tukey post hoc tests, all pairwise comparisons  $p < .001$ ). We pooled the males from the reference plot and both the original and the new occupants in the field experiment together for the ANOVAs based on the photo-based CIELAB color scores.

#### 3.2 | Establishment stage

The by-eye color morph frequency of males that established in the vacant territory (the “new occupants”) is 25% red, 23% blue, and 52% intermediate. This proportion was not significantly different from the morph frequency of the reference plot males (two-sample chi-square test:  $\chi^2 = 0.41$ ,  $df = 2$ ,  $p = .816$ ). The result did not change when we use the adjusted population morph frequency (see *Statistical Analyses*; two-sample chi-square test:  $\chi^2 = 0.77$ ,  $df = 2$ ,  $p = .681$ ). When we compared the photo-based color score between the new occupants of the vacant territories and males from the population reference plot, we similarly found no difference in the two hue-related scores (two-sample *t* test, square-root transformed to meet normality assumptions, green-red index *a*:  $t = 1.001$ ,  $p = .315$ ; blue-yellow index *b*:  $t = 1.04$ ,  $p = .300$ ; Figure 3a,b). However, the new occupants were significantly brighter (have higher *L* scores) than the reference plot males (two-sample *t* test, log-transformed to meet normality assumptions,  $t = 2.38$ ,  $p = .019$ ; Figure 3c). Body size was not different between the new occupants and the reference plot males (two-sample *t* test,  $t = 0.28$ ,  $p = .779$ ; Figure 3d).

#### 3.3 | Contest stage

In the GLM modeling the likelihood of the original occupant winning, we detected no significant effects of the photo-based color score (*L*, *a*, and *b* index differences) or SVL difference, suggesting no effect of color or body size on contest outcome (Table 1). However, we found a significant effect of residency status: The original occupant was more likely to win the contest than the new occupant (Table 1; exact



**FIGURE 3** Photo-based color score (a–c) and body size (d) comparisons of the new occupants (New) and males from the population reference plot (Ref)

binomial test,  $n = 19$ ,  $p = .004$ ). Of the 19 contest pairs, 16 (84%) were won by the original occupant (Figure 4).

## 4 | DISCUSSION

Males of divergent phenotypes are often unequal in competitive ability, and such asymmetric dominance can mediate ecological and evolutionary processes when the divergent populations meet. However, despite variation in aggressiveness among several allopatric color morphs in *O. pumilio* (Rudh et al., 2013), our study showed no evidence of asymmetric dominance between the sympatric red and blue morphs in our focal population. Brighter males (of both red and blue morphs) were able to establish in a vacant territory more so than duller males. This is consistent with previous findings in another *O. pumilio* color morph that brightness might be indicative of RHP, with brighter males being more aggressive and eliciting stronger aggressive responses from their rivals (Crothers, Gering, & Cummings, 2011; Crothers & Cummings, 2015). However, brightness did not predict higher success in winning the subsequent staged territorial

contest in our experiment. Body size, a major determinant in fighting ability in anurans (Dyson et al., 2013) and many other taxa (Arnott & Elwood, 2009; Hsu et al., 2006), was also not predictive of success in *O. pumilio* territorial competition. This adds to the evidence in several previous studies (Crothers & Cummings, 2015; Pröhl & Hödl, 1999), suggesting that body size is not a determinant of territory acquisition in *O. pumilio*. It is possible that body condition, instead of body size, was a better predictor of contest outcomes (as found in Meuche et al., 2012); however, we did not weigh the males and were not able to test this hypothesis. Comparatively, in 84% of the staged contests, the original occupant won against the new occupant, revealing a strong prior residence effect. Therefore, contrary to our prediction, the male aggressiveness divergence that has been observed in allopatry seems insufficient to mediate social dominance and drive trait evolution when *O. pumilio* morphs meet in sympatry.

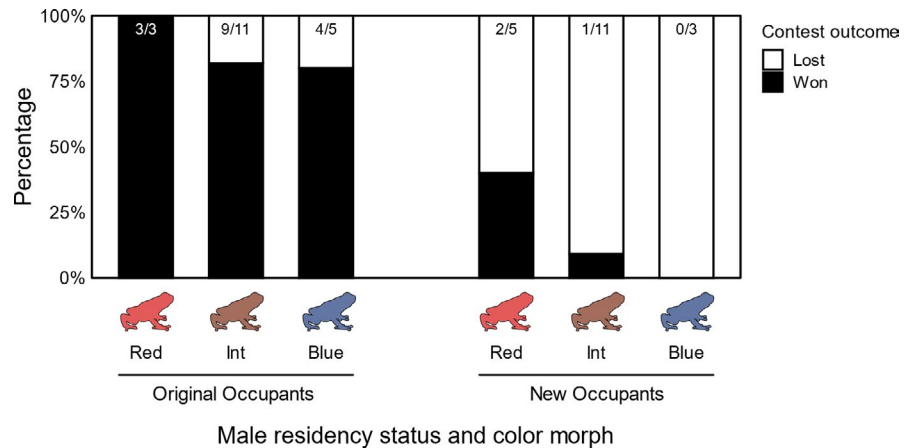
Several potential experience-based mechanisms could contribute to the original occupants winning the majority of the contests. As observed in taxonomically diverse animals, territorial residents frequently win against intruders in dyadic contests, even when the intruder has a similar fighting ability to the resident (prior residence effect; Davies, 1978; Kokko et al., 2006). In laboratory enclosures, *O. pumilio* show prior resident effects within a mere 7 days of acclimation, and the effect remains even after a 6-day removal period (Baugh & Forester, 1994). In our experiment, we manipulated both males in the staged contest pair to perceive the location as their territory. However, the difference in time spent occupying the territory (presumably longer for the original occupant) may have led to asymmetry in knowledge of the territory resource value, and further influenced the motivation to persist or retreat in a contest (Davies, 1978; Forester et al., 1993). Furthermore, we detected strong prior residence effect even though the original occupants were removed from their territory and kept in captivity for much longer (5–38 days) compared with the new occupants (1–11 days). The persistence of the prior residence effect for up to several weeks demonstrates the impressive spatial cognition and memory of territorial *O. pumilio* males. Previous interactions

**TABLE 1** Generalized linear model evaluating the effect of residency status,  $L$  index (brightness) difference,  $a$  index (green-red) difference,  $b$  index (blue-yellow) difference, and snout-vent length (SVL; body size) difference between the two males on the likelihood of winning the territorial dispute

Parameter	df	$\beta \pm SE$	$LR\chi^2$	$p$
Intercept <sup>a</sup>	1	$4.57 \pm 3.14$	14.941	<.001
$L$ (brightness)	1	$0.03 \pm 0.50$	0.004	.951
$a$ (green-red)	1	$0.58 \pm 1.17$	0.684	.408
$b$ (blue-yellow)	1	$-0.10 \pm 1.42$	0.006	.940
SVL	1	$0.73 \pm 0.95$	0.690	.406

<sup>a</sup>Significance testing for the effect of the intercept corresponds to the effect of residency status on contest outcome. See section 2.6.

**FIGURE 4** Outcome of the 19 staged contests in which we successfully identified a territorial winner. Of the 19 contest pairs, 16 were won by the original occupant. Bars were separated by male residency status (original or new occupant) and color morph (categorized by eye as red, intermediate, and blue). Numbers at the top of each bar indicate the number of the males that won the contest (numerator) out of the total number of males (denominator) for each category



with a recognized rival may also influence an individual's willingness to engage in or escalate a contest, either due to lowered competition risk (e.g., of a neighbor territorial owner: dear enemy effect; Temeles, 1994; Ydenberg et al., 1988) or to avoid the unnecessary cost of escalated fights in an established dominance hierarchy (Barnard & Burk, 1979; Tibbetts & Dale, 2007). If the new occupant was a neighbor territorial male or satellite male at the location (as opposed to a floater wandering in from afar), he might have avoided fighting with a previous territorial owner that he had lost to before. However, contrary to this hypothesis, *O. pumilio* do not seem to visually or acoustically recognize familiar individuals (Bee, 2003; Gardner & Graves, 2005), and a previous losing experience with a known intruder does not diminish prior residence effects in the laboratory (Baugh & Forester, 1994). Lastly, the original occupants may simply be better fighters than the new occupants in certain attributes that we did not characterize. Due to the correlative nature of this field study, residency status may be confounded with fighting ability ("self-selection," Bégin et al., 1996). However, because both the original occupant and the new occupant were both self-selected winners that arose from the pool of males inhabiting the area, we expect this effect to be comparatively small.

Studies that have examined the relative importance of intrinsic fighting ability (RHP; as inferred from body size, weaponry, or status badge) and territorial residency have found mixed results across species and trait categories. Body size asymmetry, when sufficiently large, is often more important than prior experience or residency status in determining contest outcomes (reviewed in Hsu et al., 2006; Kokko et al., 2006). The relative importance of body coloration and prior residency is more variable. Prior residency overwhelms coloration in determining contest outcomes in some species (e.g., tree lizard: Zucker & Murray, 1996; wall lizard: Sacchi et al., 2009; Italian ruin lizard: Titone et al., 2018), but was found to be secondary or negligible in comparison to coloration in others (e.g., red-flanked bush robin: Morimoto et al., 2006; viviparous lizard: Martin et al., 2016). Perhaps because most frogs rely on acoustic signals for communication, there is comparatively less research on the effect of coloration on male–male competition for this group (Rojas, 2017). In *O. pumilio*, our study suggests that asymmetries that stem from prior experiences and residency status override the effects of color and body size in mediating male territoriality. RHP asymmetries, when

masked by additional factors such as prior residency, may have limited influence in determining dominance of competing lineages or species in the wild (Wiebe, 2016). With no significant asymmetric dominance between red and blue *O. pumilio* males in nature, male–male territorial competition alone seems unlikely to exert selection on coloration in this red–blue polymorphic population. Whether the same rule applies for all *O. pumilio* color morphs or other color polymorphic poison frogs requires further investigation. In addition, limited by small sample sizes and the correlative nature of a field experiment, we were unable to test for an interaction between male coloration and residency status. As such, it is difficult to interpret the effect, or the lack thereof, of prior residency on trait evolution and population divergence. An interesting future direction would be to explicitly test for such interactions to further understand the role of prior residence effects on *O. pumilio* color evolution.

Broadly, our study emphasizes the discrepancy between aggressiveness acquired from standard behavioral assays and its manifestation in an organism's natural social context. It also highlights the need to incorporate information on residency status or prior contest experience into studies of trait divergence, where the flashier focal trait is usually the only target in hypothesis testing.

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#### DATA AVAILABILITY STATEMENT

The dataset of this study and the code files for statistical analyses have been deposited in Figshare (10.6084/m9.figshare.12915395).

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