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Original Article

Black and orange coloration predict success during male–male competition in the guppy

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Investigating how intrasexual competition and intersexual mate choice act within a system is crucial to understanding the maintenance and diversity of sexually-dimorphic traits. These two processes can act in concert by selecting for the same trait, or in opposition by selecting for different extremes of the same trait; they can also act on different traits, potentially increasing trait complexity. We asked whether male–male competition and female mate choice act on the same male traits using Trinidadian guppies, which exhibit sexual size dimorphism and male-limited color patterns consisting of different colors arranged along the body and fins. We used behavioral assays to assess the relationship between color and competitive success and then compared our results to the plethora of data on female choice and color in our study population. Males initiated more contests if they were larger than their competitor. Males won contests more often if they had more black coloration than their competitor, and the effect of black was stronger when males had less orange than their competitor. Additionally, males won more often if they had either more structural color (iridescence) and more orange, or less structural color and less orange than their competitor, suggesting multiple combinations of color traits predict success. Females from our study population exhibit a strong preference for more orange coloration. Thus, traits favored in male contests differ from those favored by intersexual selection in this population. These results suggest that inter- and intrasexual selection, when acting concurrently, can promote increased complexity of sexually selected traits.

Key words: **animal behavior, color polymorphism, intersexual mate choice, intrasexual competition, male contests, *Poecilia reticulata*.**

INTRODUCTION

Elaborate secondary sexual traits have long perplexed evolutionary biologists. Darwin proposed sexual selection theory to explain how and why these conspicuous, and seemingly costly, traits persist in the natural world (Darwin 1871). He suggested that elaborate traits evolve to enhance individual mating success by either aiding rivals during intrasexual competition, or by attracting mates during intersexual mate choice (Darwin 1859, 1871). Early studies of sexual selection mainly focused on one of these processes at a time, rather than considering their potential interactions. Historically, traits used during intrasexual competition were thought to facilitate intersexual mate choice (Andersson 1994). More recent investigations indicate that the two forces can sometimes counteract each other, however (Howard et al. 1997; Moore and Moore 1999; Kangas and Lindström 2001; Lopez et al. 2002; Sih et al. 2002; Bonduriansky and Rowe 2003; Candolin 2004; Wong and Candolin 2005). Understanding when and how intrasexual competition and intersexual mate choice act, and potentially interact, within systems has important implications for understanding prominent questions in evolutionary biology, such as elaborate trait evolution, the maintenance of genetic variation, the evolution of sexual dimorphism, conflict between the sexes, and speciation (Lande 1980; West-Eberhard 1983; Arnqvist and Rowe 2005; Hunt et al. 2009; Servedio and Boughman 2017).

When a species exhibits overt mate choice, the effects of intrasexual competition are often assumed to be of minor importance and/or they are controlled for with statistical analyses or experimental design (Hunt et al. 2009). Nevertheless, in systems that exhibit both intrasexual competition and intersexual mate choice, it is rare that the two act independently of one another (Moore and Moore 1999). The same trait, or an overlapping set of traits, can be important in both competition and choice outcomes (Qvarnström and Forsgren 1998; Wong and Candolin 2005). When traits used to signal male competitive ability also attract females (Berglund et al. 1996; Candolin 1999), intrasexual competition reinforces mate choice and increases total selection on that trait (Zimmerer and Kallman 1988; Morris et al. 1995). For example, during combat, male green swordtails (*Xihophorus helleri*) with longer swords outcompete males with shorter swords, and females show strong

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preferences for longer sworded males (Benson and Basolo 2006). Such cases, where intrasexual competition and intersexual mate choice act on the same trait in the same direction are prevalent in the literature (reviewed in Hunt et al. 2009). In general, when females receive direct and/or indirect benefits from choosing males that are successful during intrasexual competition, female mate choice and male–male competition are expected to operate in the same direction (Berglund et al. 1996; Wiley and Poston 1996).

Intrasexual competition and intersexual mate choice do not always act in concordance, however (Burley 1981). They can act on the same traits, but in opposing directions (Wong and Candolin 2005; Qvarnström et al. 2012). For example, in a species of water boatman (*Sigara fallen*), small foreleg tarsals are favored during competition, but females show stronger preferences for large tarsal segments (Candolin 2004). In the yellow-browed leaf warbler (*Phylloscopus inornatus*), the size of male color patches preferred by females differs from the patch sizes exhibited by dominant males during male–male competition (Marchetti 1988). In these instances, conflicting selection was suggested to promote genetic variation in sexually-selected traits. Strong directional selection should reduce the genetic benefits of mate choice by fixing beneficial alleles, yet opposing intrasexual competition may slow fixation and allow phenotypic diversity to persist for longer or at higher levels than if selection operated in the same direction (Roff 1997; Candolin 2004). Additionally, when partially overlapping sets of traits signal different types of information to mates (e.g., overall condition or levels of parasitism) and rivals (e.g., competitive ability), sexual selection is predicted to increase overall trait complexity, where "complexity" refers to the number and arrangement of different traits in a single individual, e.g., the number of specific colors and their patterning. Although this hypothesis is intuitive, it has rarely been tested (Møller and Pomiankowski 1993; Johnstone 1996; Chen et al. 2012).

Sex-specific coloration is a well-known example of a secondary sexual characteristic used during intrasexual competition and intersexual mate choice across diverse taxa (Kodric-Brown and Brown 1984). Male color may indicate dominance status to rival males or enhance attractiveness to females (Endler 1980; Smith and Harper 2003; Marty et al. 2009). Orange, red, and black are the most commonly studied colors in sexual selection research (Jawor and Breitwisch 2003), in part because these colors are conspicuous to humans. Reddish colors are sometimes, but not always, derived from dietary carotenoids, which has spurred research focusing on the role of these colors as honest signals of mate quality (Andersson 1982; Kodric-Brown and Brown 1984; Pomiankowski 1987; Hasson 1989; Kodric-Brown 1989). Eumelanin-based colors (black and brown) have also received a great deal of attention, in part because the molecular pathway that produces eumelanin is well described, and some mechanisms of eumelanin production have been proposed to generate extensive pleiotropy, including effects on mating behavior and aggression (reviewed in Ducrest et al. 2008; Roulin et al. 2011; but see Kraft et al. 2018; Massey et al. 2019). These arguments suggest that mate choice should often be affected by carotenoid-based coloration, whereas intrasexual competition should be influenced by melanic coloration. However, few studies have examined the role of the same suite of color traits in both components of sexual selection.

Here, we assessed whether male–male competition and female's mate choice select for the same or different color traits in Trinidadian guppies (*Poecilia reticulata*). Guppies have complex, male-limited color patterns (Winge 1927; Houde 1997) that differ among populations and are also highly polymorphic within populations (Endler and Houde 1995; Brooks and Endler 2001a; Hughes, et al. 2005). Male guppy color patterns typically consist of carotenoid and pteridine pigments (orange, red, yellow), melanin (black and brown) pigments, and patches of structural colors including white and iridescent blues, violets, greens, and silver (Kodric-Brown 1985; Grether et al. 2001; Kemp et al. 2008). Female guppies exhibit strong mate choice for male color patterns (Hughes et al. 1999, 2013; Hampton et al. 2009; Mariette et al. 2010; Valvo et al. 2019). In some guppy populations, females prefer males with large amounts of orange coloration (Endler 1983; Kodric-Brown 1985, 1989; Houde 1987b; Long and Houde 1989; Houde and Endler 1990; Valvo et al. 2019). Similarly to orange, some populations prefer black coloration (Brooks and Caithness 1995b; Endler and Houde 1995; Brooks 2002), but some populations show no preference (Nicoletto 1993; Brooks and Caithness 1995a; Kodric-Brown and Nicoletto 1996; Brooks and Endler 2001a, 2001b), or even antipathy towards black (Endler and Houde 1995). Unlike orange, black expression can be plastic and may be under neuronal and hormonal control (Fujii 2000; Aspengren et al. 2003; Gibson et al. 2009); these physiological color changes are important to consider when interpreting the significance of black during mating events.

The role of guppy color pattern in mate choice has been extensively studied for decades, yet little is known about the importance of color during male–male competition despite the propensity for males to partake in intrasexual competitive behaviors (Kodric-Brown 1993; Houde 1997; Price and Rodd 2006; Daniel and Williamson 2020). Male–male competition in guppies rarely involves direct physical contact; however, males often compete over optimal position relative to females, both when pursuing females and when attempting courtship displays (Farr 1980; Houde 1987, 1997; Kodric-Brown 1993; Price and Rodd 2006; Daniel and Williamson 2020). Dominant males descended from two wild populations have been reported to have more orange and structural coloration than subordinate males (Kodric-Brown 1993). Additionally, during intense courtship, males tend to expand and darken some of their black spots along the body and tail, whereas other colors remain relatively unchanged (Nayudu 1979). Although these are suggestive reports, the role of black coloration during intrasexual competition remains relatively unexplored in guppies.

We sought to determine the role of color pattern, including the differences in the extent of orange and black coloration expressed between rival males, during intrasexual competition in guppies derived from a Paria Tributary in Trinidad, and to relate our findings to a large body of mate choice literature on this specific population. Compared with other populations, males from this tributary have large areas of orange pigmentation (derived from both carotenoids and pteridines) across their bodies, along with smaller areas of black (eumelanin), and structural color (iridescent blue, green, and silver) (Endler 1978; Houde 1997). Female preference for orange is especially strong in guppies from this population (Houde 1987, 1994; Long and Houde 1989; Endler and Houde 1995; Houde and Hankes 1997; Graber et al. 2015). Females from this tributary were found to be indifferent to black (Endler and Houde 1995), and no significant associations between black coloration and male–male competition in the form of dominance behaviors (chases and gonopodium nips) have been reported in this population (Kodric-Brown 1993).

We also examined the importance of male size during intrasexual competition because size is often an indicator of competitive ability (Smith and Brown 1986; Andersson 1994; Ligon 1999). The role of male size in guppy mate choice is variable across populations; females from some populations make mating decisions based on male size (Bischoff et al. 1985; Reynolds et al. 1993; Endler and Houde 1995; Magellan et al. 2005; Auld et al. 2016), whereas females from other populations show no association between choice and size (Endler and Houde 1995; Schwartz and Hendry 2007). Females from the Paria Tributary have been documented to prefer males with small body size but large caudal fins (Endler and Houde 1995). To our knowledge, the role of size during male–male competition in this population has not been previously explored.

We asked whether aspects of male color pattern and size predict male success during competitive contests. Specifically, we tested whether male body size along with orange, black, and structural colors predict initiating and winning male–male competitive contests. Male guppies often engage in multiple bouts of competitive interactions with the same rival, rather than one-off dyadic contests (see Methods). We, therefore, investigated the effects of size and color expression on the number of competitive bouts that a male initiated and his rate of winning bouts. Because there were two males in each trial, we assessed color and size differences between the male that was deemed the overall winner of the competition and the male that was deemed the loser ("winner" males were those that won >50% of interactions over the length of an entire trial). There was still variation in initiation and success rates among winners, so we investigated the effects of size and color differences on the rate of initiating and winning bouts among overall winners. We then asked if these results suggest that overlapping sets of traits are associated with male success during both female mate choice and male–male competition. Given the documented female preferences in this population, we predicted that, if female choice and male–male competition act in concordance, then males with increased amounts of orange, small bodies, and large caudal fins should be more successful during competitive bouts. However, if female choice and male–male competition act in opposition, we predicted that males with large bodies, small caudal fins, and colors other than orange should be more successful during competitive bouts. Because correlative evidence suggests that eumelanin is important during aggressive encounters, we hypothesized that increased amounts of black should reflect overall male competitive success.

METHODS

Ethics statement

All procedures using live animals were approved by the University of Toronto's Animal Care Committee (protocols 20010160 and 20009555) and complied with the Canadian Council of Animal Care.

Animal husbandry

Fish used in this experiment were laboratory-reared at the University of Toronto and descended from wild-caught guppies from the "Houde" Tributary of the Paria River in Trinidad (Trinidad National Grid System: PS 896 886). We chose this study population because mate choice, reproductive behaviors, and male coloration have been well-documented (e.g., Houde 1987, 1988a, 1988b, 1992, 1997; Long and Houde 1989; Daniel et al. 2019; Valvo et al. 2019). Fish were maintained on a 12:12 h light:dark cycle at 26 °C and fed TetraMin fish food in the mornings and nauplii larvae (*Artemia salina*) in the afternoons. Fish were moved among 54 L stock tanks (60 × 30 × 30 cm^3) every one to –two generations to minimize inbreeding.

Behavioral trials

Guppies are live-bearers with internal insemination (Liley and Stacy 1983; Houde 1997). During courtship, adult males spend a majority of their time following females and performing courtship displays (Baerends et al. 1955; Houde 1997). The primary competitive behavior exhibited by males in our study population is interruptions, which occur when two males are simultaneously pursuing a female (Price and Rodd 2006; Daniel and Williamson 2020). An interruption consists of the trailing male darting in front of the leading male, placing himself directly behind the female, and usurping access to her gonopore (i.e., genital opening). Interruptions often occur in bouts where two males rapidly cut off one another in quick succession. The male that performs the last interruption in a bout ends up with access to the female, and therefore can be considered the "winner" of that bout. Interruptions have been reported to reduce the losing male's opportunity to attempt mating (Price and Rodd 2006; Daniel and Williamson 2020). Therefore, interruptions are likely an important component of intrasexual selection in this population. During this male–male interaction, the female is typically foraging or swimming about the tank, not fleeing from the competing males or overtly influencing the outcome of the interruptions. After the competitive interaction, the female may exert choice by deciding whether or not to mate with the remaining male.

We quantified two features of interruption behavior: 1) the number of times each male initiated a bout of interruptions (by performing the first interruption in that bout), and 2) the proportion of bouts each male won (by performing the last interruption in that bout, thus gaining access to the female). Each bout consisted of one or more interruptions, and we considered interruptions to be part of the same bout if there were less than 2 s between sequential interruptions. We used a 2-s cutoff because this was the smallest time interval between interruptions in which we observed one or both of the males performing other behaviors (e.g., courtship or mating attempts), indicating that a 2-s interval allows time for males to switch to performing other kinds of behaviors.

To determine the association between male morphology and performance during male–male competition, we quantified male competitive behaviors performed in mixed-sex groups. We observed freely-interacting groups of five guppies – —two males and three females – providing a female-biased sex ratio similar to that observed in natural guppy populations (Rodd et al. 1997; McKellar et al. 2009). Guppies are capable of individual recognition (Griffiths and Magurran 1997a, 1997b; Mariette et al. 2010; Daniel and Rodd 2016), and individual familiarity can alter male–male interactions through the establishment of dominance relationships (Gandolfi 1971; Astrid Kodric-Brown 1992; Bruce and White 1995). To prevent individual familiarity from influencing male–male interactions in our experiment, the two males used in a given trial were taken from separate stock tanks that were visually isolated from one another. Similarly, relatedness has been found to affect levels of competition among male guppies (Daniel and Williamson 2020). Consequently, for each trial, we used two males that were at most second cousins ($r \leq 0.03125$). All males were sexually mature at the time they were used in the experiment, and, because they were drawn from mixed-sex tanks, had experience courting females and competing with rival males. We used virgin females because female guppies are receptive as virgins and during a 2 to 3-day period after parturition but are not receptive once gravid (Houde 1997). Female receptivity can affect male motivation to pursue and compete for females (Guevara-Fiore et al. 2010). Virgin females were derived from female-only tanks that were visually-isolated from male tanks to ensure they were unfamiliar with all males used in the same trial. For the behavioral trials, we first placed the females in the 54 -L observation tank ($60 \times 30 \times 30$ cm^3) and allowed them to acclimate for 30 min before starting the trial. The trial began as soon as the two males were simultaneously added to the tank, therefore males did not have any opportunity to establish dominance relationships before our observations. We live-scored male competitive behaviors immediately after the addition of males to the tank for 30 min using JWatcher v 1.0 (Blumstein and Daniel 2007). All trials were conducted between 9:30 and 11:30 AM, when guppies are sexually active, to control for diel variation in reproductive behaviors (Houde 1997). We fed fish in their home tanks 30 min before transferring them to the observation tank to discourage foraging behavior during the trial. To eliminate potential observer effects, all trials were scored by the same person (M.J.D.). We observed a total of 72 trials (144 males) and excluded five trials in which males never engaged in interruption behavior. Males and females were never re-used across trials. All females were approximately the same age and of similar size.

Male phenotypes

Male size and color data were collected from digital images (Nikon Coolpix 950) taken immediately after the behavioral trials. Each male was lightly anesthetized in buffered MS-222 (ethyl 2-aminobenzoate methane sulphonic acid salt, Sigma-Aldric, St. Louis, MO, USA), and then placed laterally on a gray plastic surface with the left side-body facing up. The dorsal and caudal fins and the gonopodium of each male were carefully spread away from the body using a fine-tip paintbrush, and a metric ruler was placed beside each male. Fish were illuminated by two LEDs (MSC-208 Cold LED). Before taking the photo, a white plastic color standard was used to set the white balance, correcting any minor variation in lighting conditions between photos.

We quantified three aspects of male size: total lateral area (tip of the snout to the anterior end of the caudal fin), lateral body area (tip of the snout to the posterior end of the caudal peduncle), and dorsal plus caudal fin area for each male (Figure 1). Using ImageJ. v1.45s, we traced the appropriate regions of the fish. Within each region, we measured total orange, black, and structural color area separately by outlining color areas with the freehand tool. Our measurement of black included permanently expressed black spots and facultatively expressed "fuzzy" black (Baerends et al. 1955). Anesthetic treatment is known to increase the expression of black in guppies (Price et al. 2008); thus, the amount of black captured in our photos should reflect maximal expression exhibited during competitive interactions. Structural color areas (iridescent blue, green, and silver) are best captured when photos are taken at various angles along orthogonal axes depending on the color of interest (Kemp et al. 2008), therefore it is possible that our static photos did not accurately capture true iridescence. The standard length of each fish was measured by taking the distance between the tip of the snout and the posterior end of the caudal peduncle and was highly correlated with all area measurements ($r > 0.77$), except dorsal fin area ($r = 0.48$) (Supplemental Figure S1). We, therefore, used the three measures of body area, but not standard length, in our subsequent analyses to keep body measurements on the same scale as our color measurements.

Statistical analysis

To determine the importance of color pattern and size on overall success during male–male competition, we performed two separate analyses to address the following questions: what aspects of male color and size best predict 1) how often a male initiates a bout of interruptions (i.e., "initiations"), and 2) the probability that a male wins a bout (i.e., "successes")?

For both analyses, we examined the extent to which differences in coloration and size between rival males were associated with male competitive behavior or outcomes. We wanted to know whether particular regions of the fish were better predictors of male competitive success than others, therefore we compared three model types (model specifics below) (Figure 1). In the first model ("Total"), the predictors were the differences between males in body and color area, where both body and color area were measured over the total lateral area of the fish (body plus fins). In the second model ("Body"), the predictors were based on analogous metrics, but those metrics of body and color were measured over only the body area of the fish (fins excluded). In the third model ("Fins"), the predictors were based on body size and color measured over only the dorsal and caudal fin areas. We assessed body and fin models separately as previous work has shown that caudal fin size is preferred by females in this population (Endler and Houde 1995). We used color and size differences between males (rather than absolute measures of color and size) as predictors, as is standard in the contest literature (Hardy and Briffa 2013). Calculating differences allowed us to ask whether a male's competitive behavior and competitive outcomes were dependent on his color and size relative to his rival's color and size.

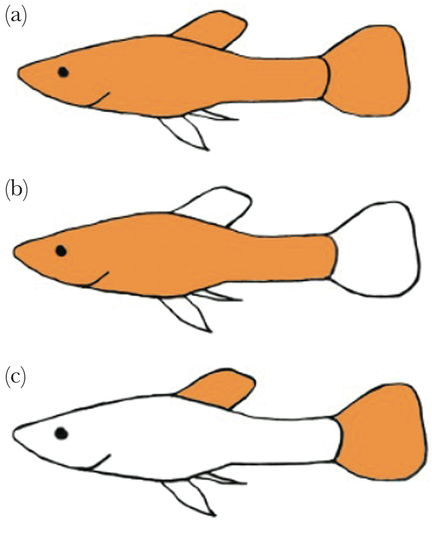

Figure 1
Three competing models were used in both initiation and win analyses to determine what aspects of phenotype along particular regions of the male were most associated with male competitive outcomes (shaded): (a) total lateral area ("Total"), (b) body area ("Body"), and (c) dorsal and caudal fin areas ("Fins").

Table 1

Results of AICc model comparison for initiation and win data

Analysis		Model	*K*	AICc	ΔAICc	*w*
Initiations	**1**	**Total (s)**	**12**	**399.24**	**0.00**	**0.55**
	2	Body (s)	12	399.82	0.58	0.41
	3	Fins (s)	14	404.48	5.25	0.04
	1	**Total (l)**	**12**	**381.66**	**0.00**	**0.63**
	2	Body (l)	12	382.75	1.09	0.37
	3	Fins (l)	14	395.21	13.55	0.00
Wins	1	Total	11	327.52	16.9	0.00
	2	**Body**	**11**	**310.64**	**0.00**	**1.00**
	3	Dorsal and caudal	12	338.84	28.2	0.00

K = estimated model parameters, *w* = model weights. Initiation analyses show model outputs using the number of initiations performed by successful males (s) and loser males (l). Best fit models are shown in bold.

For each body region model, the measured male colors included difference in orange, black, and structural color area, as well as difference in size area. Successful males were defined as the male that won >50% of the bouts in the trial, and the other male was designated the loser male. For trials in which males tied ($n = 4$), we randomly selected one male as the successful male. Results for a data set that excluded these four ties were qualitatively the same (same models selected, same effects significant). We, therefore, report only the results for the complete data set. To standardize the direction of these measures, we calculated differences by subtracting loser male color and size from successful male color and size in any given trial. We scaled all predictor variables to have a standard deviation of 1. Each model was assessed for overdispersion, multicollinearity, and variance inflation. Our predictors were approximately normal and homoscedastically distributed, therefore no transformation was applied. For model selection, we used AICc comparison and calculated cumulative model weights using the aictab function from the AICcmodavg package v 2.2-2 in R v 3.6.2 (R Core Team 2019). We then conducted likelihood ratio tests on the best fit model to assess the significance of fixed effects.

To determine which color and size features best predicted the number of initiations performed during a competitive bout, we used generalized linear models with a negative binomial distribution (log link function) using glm.nb from the MASS package v 7.3-51.4. Because there were two males in each trial, we used the number of initiations performed by the male who ultimately won the competition (i.e., successful males) as our response variable to avoid pseudoreplication. We used the following predictors: difference in size, difference in color (orange, black, and structural) between rival males, and all two-way interactions. In addition, we wanted to know whether the same color and size features that are associated with the number of initiations performed by successful males were also important for loser males. We, therefore, repeated the analysis using the number of initiations performed by the loser males as the response variable. We also tested whether winner and loser males differed in how often they initiated bouts of interruptions by using a paired permutational *t*-test to compare number of interruptions between these two sets of males (*RVAideMemoire* v 0.9-80). We performed a two-sided test with 10,000 permutations. To determine which color and size features best predicted the likelihood that a male won a competitive bout, we used generalized linear models with a binomial distribution (logit link function) using glm from the stats package v 4.0.4. The response variable was the proportion of wins (number of wins/bout) won by the successful male, weighted by the total number of bouts in a trial. Our predictor variables were the same as those described above.

RESULTS

Initiations

Successful males and loser males did not differ significantly in the number of times they initiated bouts of interruptions (permutational *t*-test: df = 71, $t = 0.709$, $P = 0.512$). The number of bouts initiated by the successful male was best predicted by color and size differences measured over the total lateral area of the fish ("Total"); however, color and size differences based only on body measures without fins ("Body") were nearly as good at predicting bout initiations as those based on total area (Table 1). Both of these models fit the initiation data significantly better than the model based only on measures of dorsal and caudal fins. In both models, the number of initiations performed by the successful male was predicted by his size (lateral area) relative to that of his competitor (Table 2 "Total"; Supplementary Table S1 "Body"). Sizes were calculated as differences (successful male—loser male) and scaled to have a standard deviation of 1; for these data, the mean difference in total body size between successful males and loser males was +5.16 mm^2 and 1 SD equated to 16.7 mm^2 (Supplemental Figure S2). Therefore, successful males were larger than their competitors on average, and the number of bouts initiated by the successful male was positively associated with his size advantage (indicated by a positive regression estimate, Table 2, Figure 2a). No other effects in the model were significantly associated with the number of bouts initiated by the successful male.

Additionally, we wanted to know whether the aspects of color and size important for the number of initiations by successful males were the same as those important for the loser males. The number of initiations performed by a loser male was best predicted by the "Total" model. In the "Total" model, the number of bouts initiated by the loser male was predicted by the difference in orange area between the rivals (Table 2). Loser males had less orange on average (Supplemental Figure S3); the mean difference in total orange area between successful males and losers was +0.53 mm^2, and 1 SD equated to 3.05 mm^2. Surprisingly, however, the number of bouts initiated by the loser males increased as their amount of orange relative to their rival decreased (Table 2, Figure 2b). No other effects in the model were significantly associated with the number of bouts initiated by the loser male.

Successes

The proportion of bouts won by the successful male was best predicted by color and size differences measured over the lateral body area of the fish ("Body"). This model fit the data significantly better than a model based on total lateral area and a model based on dorsal and caudal fin area of the fish (Table 1). In this model, the proportion of bouts won was associated with effects of black body coloration, and significant interaction effects involving differences in black, orange, and structural coloration (Table 3). Successful males had more black on average (Supplemental Figure S4; the mean difference in black body area between successful males and losers was +1.58 mm^2, and 1 SD equated to 2.20 mm^2), but this effect was modified by differences in orange coloration. Figure 3 illustrates the association between the proportion of bouts won and differences between rivals in black and orange body color. A male tended to win a higher proportion of his bouts if he had more black than his rival, and this association was stronger when the male had less orange than his rival. In addition, orange and structural colors interacted in their effects on the proportion of bouts won. Figure 4 illustrates the relationship between the proportion of bouts won and differences between rivals in orange and structural colors. Two combinations of orange and structural color differences predicted a high proportion of wins: successful males had either (1) more structural color and more orange or (2) less structural color and less orange than their competitor. Supplemental Figure S5 shows comparison images of representative experimental males with varying amounts of orange, black, and structural colors.

Table 2

Results of the "Total" GLM for the number of initiations performed by successful male and by the loser male

Initiation Data	Effect	Estimate	Std. error	LR χ^2	*P*-value
Successful males	Orange	−0.01	0.08	1.85	0.174
	Black	0.08	0.08	0.88	0.347
	Structural	−0.07	0.08	0.73	0.393
	Size	**0.19**	**0.08**	**6.12**	**0.013**
	Orange × black	0.05	0.05	0.96	0.325
	Orange × structural	0.13	0.07	3.59	0.058
	Black × structural	−0.09	0.12	0.60	0.439
	Orange × size	−0.01	0.09	0.01	0.904
	Black × size	0.02	0.07	0.10	0.754
	Structural × size	−0.01	0.10	0.01	0.910
Loser males	**Orange**	**0.16**	**0.07**	**5.91**	**0.015**
	Black	−0.11	0.08	2.36	0.124
	Structural	0.12	0.08	1.81	0.178
	Size	−0.10	0.07	1.89	0.170
	Orange × black	0.01	0.05	0.05	0.821
	Orange × structural	0.03	0.06	0.24	0.626
	Black × structural	0.07	0.12	0.31	0.574
	Orange × size	0.03	0.08	0.16	0.686
	Black × size	−0.03	0.08	0.17	0.679
	Structural × size	0.11	0.10	1.30	0.254

Significant *P* values are bolded. Degrees of freedom = 61.

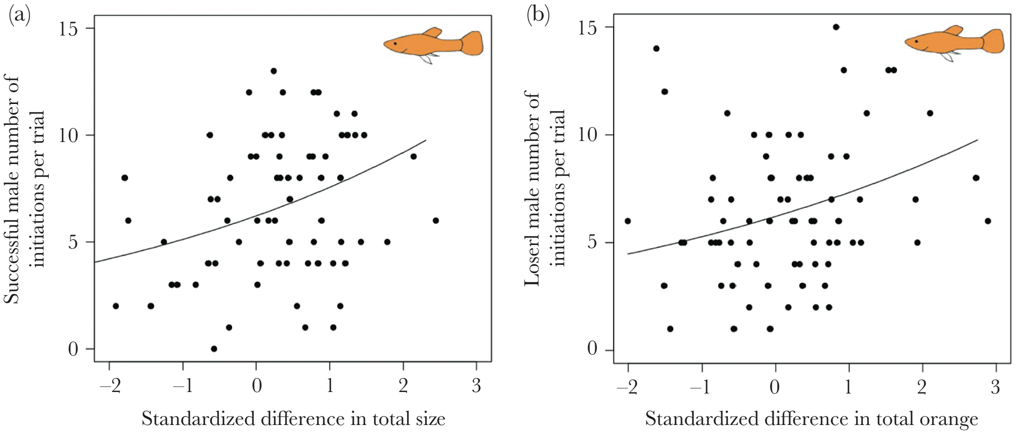

Figure 2

Scatterplot illustrating results from best-fitting "Total" model predicting number of initiations by the successful and loser males. (a) Successful males that were larger than their rival initiated more interruptive bouts; (b) loser males with less orange than their rival initiated more interruptive bouts (b). Solid points represent the raw data and black lines show the predicted values from the generalized linear model fit. Differences were calculated by subtracting loser male total size from successful male total size (mm), and then standardized (SD = 1).

Table 3

Results of the “Body” GLM for color and size differences between rivals on the proportion of bouts won by the successful male in each trial

Win data	Effect	Estimate	Std. error	LR χ^2	*P* value
	Orange	−0.16	0.09	3.43	0.064
	Black	**0.52**	**0.14**	**14.2**	**<0.001**
	Structural	0.00	0.12	0.00	0.997
	Size	−0.07	0.13	0.33	0.567
	Orange × Black	**−0.25**	**0.13**	**4.19**	**0.041**
	Orange × Structural	**0.40**	**0.15**	**7.51**	**0.006**
	Black × Structural	−0.25	0.16	2.77	0.096
	Orange × Size	0.04	0.12	0.18	0.669
	Black × Size	−0.09	0.11	0.65	0.417
	Structural × Size	−0.01	0.14	0.00	0.957

Significant *P* values are bolded. Degrees of freedom = 61.

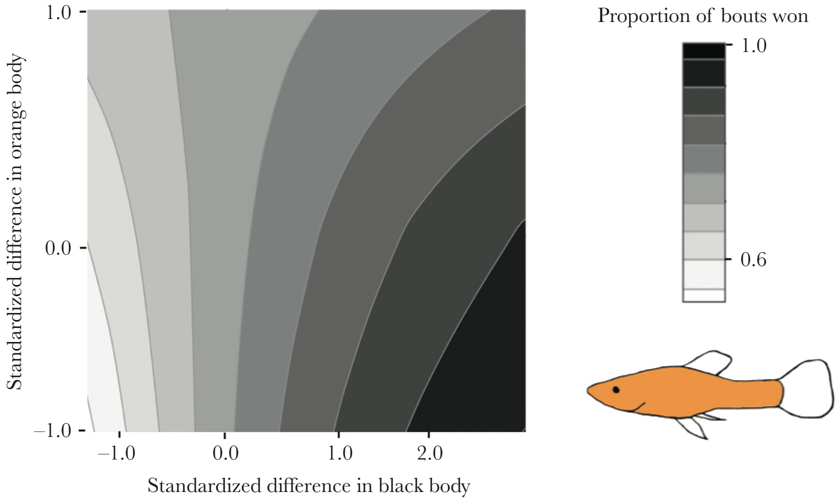

Figure 3

Contour plot depicting the association between proportion of bouts won and differences in orange and black body coloration between rivals, using results from the best-fitting “Body” model. Results are based on proportion of bouts won by the male that won > 50% of bouts; therefore, the lower bound for this proportion is 0.5. Darker regions represent cases in which successful males won a high proportion of bouts within a trial, whereas lighter regions represent cases in which successful males won a lower proportion of bouts. The proportion of bouts won increased as the amount black body color, relative to rivals, increased (indicated by the trend for contours to darken from left to right). The strength of this relationship was modified by the relative amounts of orange, with a stronger relationship for males that had less orange body color relative to their rivals (indicated by the contours changing more rapidly in the bottom half of the plot). Differences in body coloration were standardized (SD = 1).

DISCUSSION

We set out to determine if traits that are associated with success during intrasexual competition are the same as those that are important for mate choice in a population of guppies for which mate choice has been well characterized. We assayed male–male competitive behaviors and quantified male color and size using fish descended from the “Houde” Tributary of the Paria river in Trinidad. Our results suggest that males from this population initiate competitive bouts based on their size relative to rivals, and they win more bouts when they exhibit specific, and rather intricate differences from their rival in black, orange, and structural coloration. Therefore, intrasexual competition likely selects on complex features of male color pattern (specific colors and color arrangement), and perhaps male size.

As predicted, size was important during male–male competition. Specifically, for successful males, total male size was the best predictor for initiating interruptive bouts with larger males initiating more of these bouts than smaller males. In previous studies, females from this population have been reported to prefer males with larger caudal fins (Endler and Houde 1995). However, we found that fin size alone was a poor predictor of initiating competition; rather, the entire area of the fish was the best predictor, and body area (excluding fins) was nearly as good. In other guppy populations, various aspects of body and caudal fin size have been reported to be associated with female choice, but these preferences are inconsistent across studies and populations (Houde 1997), which may be a result of differences in experimental design. Here, we provide evidence that male size plays an important role in the initiation of competitive interactions and that male–male competition and female choice act on different aspects of the same trait (total size versus caudal fin size). Across Poeciliid fishes, size seems to be a significant

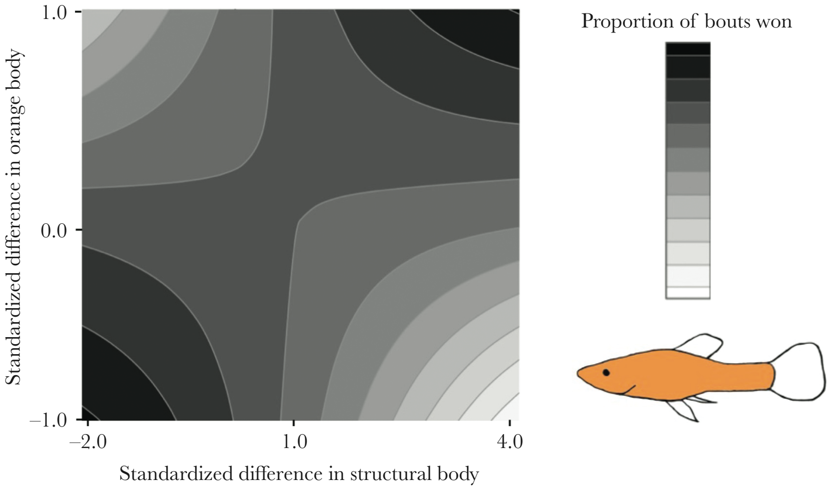

Figure 4

Contour plot depicting the association between proportion of bouts won and the interaction between structural and orange body coloration from best-fitting "Body" body. Symbols as in Figure 3. Males won more bouts if they had more structural color and more orange or less structural color and less orange than their rival.

predictor of male–male competition and/or female mate choice. In green swordtails (*X. helleri*), females exhibit a preference for males with longer swords (Basolo 1990) and longer swords outcompete shorter swords (Benson and Basolo 2006). In Panuco swordtails (*Xiphophorus nigrensis*), laboratory studies have shown females spend more time in close proximity to larger males than smaller males (Ryan et al. 1990) and larger males successfully block smaller males from gaining access to females (Morris et al. 1992). In other vertebrate taxa, larger males often have an advantage over smaller males during intrasexual competition (reviewed in Wong and Candolin 2005). Similar to our study population, female tiger salamanders exhibit a preference for males with longer tails. However, body size is important during male–male competition and larger males are more likely to interfere with mating attempts of longer-tailed males (Howard et al. 1997). If males with less-preferred traits are more likely to displace preferred males, then behaviorally dominant males might limit females' access to preferred mates (Kodric-Brown 1992; Wong and Candolin 2005).

Loser males initiated more bouts when their rival had more orange coloration and successful males won a higher proportion of those bouts if they had more black body color relative to their rival. These results suggest that the relative amount of color between competitors plays a role in male competitive success. One possibility is that loser males initiated more often with rivals expressing higher levels of orange (a color preferred by females) as a sneaking strategy. Perhaps if less-attractive males stay in close proximity to more attractive males, then they may have increased opportunities to sneak-copulate with females. In guppies, sneak mating has been reported to be influenced by many factors, including female receptivity, predation risk, male age, male body size, population density, gonopodium length, sex ratios, water turbidity, and ambient light (Endler 1987; Magurran and Seghers 1990; Magurran and Nowak 1991; Reynolds et al. 1993; Godin 1995; Rodd and Sokolowski 1995; Jirotkul 1999). Our results suggest that different individual males adopt tactics based, in part, on the phenotypes of their rivals; in other words, male guppy reproductive tactics may be a frequency-dependent function of male color patterns.

One strength of our experiment is that we asked whether male traits that were important for initiating competition were the same as those important for succeeding in those contests. Intriguingly, we found that although size was a significant predictor for initiating competitive bouts (for successful males), it was not important for predicting the outcome of those bouts (i.e., larger males initiated more often, but were not more likely to win). Male size can be a competitive cue in territorial species that need to defend resources to ensure mating success (Andersson 1994). Perhaps in weakly territorial species, such as guppies, male size is used as an initial cue to signal competitive ability, but as rival males get within close proximity size no longer matters, and other traits become more important for winning (e.g., color). We assayed interruptive bouts where one male displaces another male that is actively pursuing a female and the two jockey back and forth until one remains. From behind, the trailing male might easily assess rival size but have difficulty discerning color traits until the two are positioned side-by-side. Once the two begin interrupting one another, the trailing male might be more likely to cease fighting if the other male is expressing more visually dominant traits, such as color. Another consideration is that female guppies rely on multiple cues during mating attempts and have substantial control over mating decisions (Kodric-Brown 1993). Thus, female behavior might play a subtle role in the outcome of male competitive bouts, for example by communicating her mating preference. Future studies should attempt to disentangle the intricacies of these interactions to determine the extent to which females influence male competitive behavior and outcomes. Assessing the mechanisms that underlie male competitive decision-making would better inform us of the conditions in which competition overrides choice or choice overrides competition (see below).

Overall, males with large amounts of black on the body (although anesthetized) were more successful during competitive bouts. Black coloration has been associated with dominance and levels of aggression in a variety of species: fruit flies (Takahashi 2013), salmonid fish (Kittilsen et al. 2009), reptiles (Mafli et al. 2011), birds (Roulin et al. 2000), and mammals (Bubenik and Bubenik 1985; West and Packer 2002; Graipel et al. 2014).

Associations between expression of black and mating behaviors are hypothesized to be driven by pleiotropic effects of melanocortin pathways (Ducrest et al. 2008). However, whether melanin acts in this way in our study is unclear. In some fishes, black spots tend to expand in size as mating and competitive interactions increase (Kodric-Brown 1998). Given the plasticity of black coloration in guppies and evidence that black expression is under neuronal and hormonal control (Price et al. 2008; Tezuka, et al. 2011), more black color expression by successful males could be a consequence of dominance, and not necessarily a cause or a signal of dominance. Experiments that track color change before, during, and after competitive interactions are needed to distinguish these possibilities.

Recall that females from this population have not previously been reported to respond to black coloration, and instead exhibit a strong preference for orange. We found the association between success and black coloration was strengthened when males had less orange body coloration compared with their competitor. This result suggests male–male competition and female mate choice are not completely aligned in this population, and trait elaboration might be driven by different forms of sexual selection. More generally, color signals may be used differentially by the sexes to convey different types of information to mates and rivals, which could explain the evolution of complex male guppy color patterns (Moller and Pomiankowski 1993; Chen et al. 2012). Female preference can affect male mating success after male–male competition, potentially overriding male–male competition; however, females do not always have complete control over mating outcomes. Coercive mating attempts can circumvent female mate choice, and more aggressive male guppies have more opportunities for coercive mating attempts (Price and Rodd 2006). Thus, winning male–male competitive interactions can lead to successful matings even by unpreferred males. Godin (1995) reported that males changed their reproductive tactic from courtship displays to sneak attempts in the presence of predators, which suggests they can change their strategy in response to stimuli. It is possible that male guppies adjust their tactic in response to female interest (e.g., males with less orange may interrupt more to ensure at least some mating opportunities despite being less attractive). However, Daniel and Williamson (2020) found that female responsiveness was unrelated to the number of males pursuing her. This result suggests that male competitive decisions are not strongly related to female interest, though a more direct test is needed. To disentangle the interactions between female preference and male–male competition, future studies should assess the rate of change in male reproductive tactics and intrasexual competitive behaviors in response to female interest. Overall, we argue that male–male competition remains an important selective force on male color pattern in this system.

We also observed a complex interaction between orange and structural body color. Males won competitive bouts more often if they had more orange and more structural coloration or less orange and less structural coloration than their rivals. This result suggests that male–male competition might be generating correlational selection and multiple adaptive optima in this population. A previous analysis of a feral Australian guppy population suggested that there are at least three ways to make an attractive male guppy; each peak consisted of complex combinations of color traits that are likely preferred by females (Brooks and Endler 2001a; Blows et al. 2003), although we note that this study did not control for prior female experience of male color patterns, which is known to strongly influence female preference (Hughes et al. 1999, 2013). If females do have fixed preferences that vary individually, then this, combined with multiple competitively-successful male phenotypes, could lead to disruptive selection. Such disruptive selection could contribute to the presence of multiple male sexual ornaments and the maintenance of the extreme color polymorphism found within guppy populations.

Our results reflect those of dyadic male–male interactions, which are not necessarily representative of interactions in groups with more than two competing individuals (Lea and Ryan 2015). In nature, females are sometimes pursued by multiple males and it is possible that more than two males sometimes interrupt each other. Future work should examine more complicated networks of individuals to gain a better understanding of how male–male competition operates in various social environments. In general, studying mate choice and intrasexual competition concurrently will help determine how sexual selection drives the evolution of complex traits. Additionally, disentangling the processes driving differential selection on aspects of male size and color during intrasexual competition and mate choice could have important implications for understanding the high variability of both size and male color pattern in the guppy.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at *Behavioral Ecology* online.

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Data availability: Analyses reported in this article can be reproduced using the data provided by Guerrera, A.G. et al. (2022).

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