Assessing the dual utility of nuptial patterning in the rainbow darter (*Etheostoma caeruleum*)

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Highlights

- We tested for the dual utility of male nuptial color patterns in the rainbow darter
- We tested male success in simulated territorial contests and female choice trials
- We found a relationship between luminance contrast and success in female choice trials, but not in male-male contests

In this study, we assessed the possible dual utility of nuptial color patterns in male rainbow darters (*Etheostoma caeruleum*) in the context of male-male territorial interactions and female mate choice. Using a cohort of wild-caught male *E. caeruleum*, we conducted simulated territorial contests between pairs of males in the lab. We scored the "winner" and the "loser" of each contest based on behaviors and color changes at the end of each contest. We then performed dichotomous choice trials using the same pairs of males as stimuli and female *E. caeruleum* as focal subjects. We scored the choice trials based on female strength of preference. We assigned each male an Elo rating (a relative skill score used in zero-sum contests between pairs of players) in the male-male contests based on wins and losses, and in the choice trials based on female preference across all trials for each male. We evaluated whether success in male-male contests correlated with success in female choice trials. Finally, we measured body area, mean luminance and luminance contrast for each male, and we evaluated whether either variable correlated with success in male-male contests or in female choice trials. We did not find a statistically significant correlation between Elo ratings in the contests and those in the choice trials, although we observed a positive trend in the relationship. Neither body area nor mean luminance was significantly correlated with success in either set of trials, and luminance contrast was positively correlated with success only in the female choice trials. Our findings indicate that the vivid patterns in breeding male *E. caeruleum* may play a role in female mate choice. This study highlights the importance of evaluating the role of conspicuous nuptial characteristics in both intrasexual and intersexual selection.

KEY WORDS: mate choice, competition, intrasexual selection, intersexual selection, luminance contrast, *Etheostoma.*

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Running head: Rainbow darter nuptial patterning

INTRODUCTION

Many animals display vivid and complex color patterns during courtship. These colors are often assumed to evolve due to sexual selection, which can be conceptualized as two potentially interacting processes. Intersexual selection refers to mate choice based on preferences in one sex for certain traits in the other sex, and intrasexual selection refers to competition between members of the same sex for mating opportunities, usually through aggression or scramble competition (Darwin 1871; Moore 1990; Petrie et al. 1991; Balmford et al. 1992; Bonduriansky 2001; Kemp & Wiklund 2001; Jawor et al. 2006; Rosvall 2008, 2011; Edward & Chapman 2011; Midamegbe et al. 2011; Bonatto et al. 2013; Morales et al. 2014; Weiss & Dubin 2018; Garcia-Rosales et al. 2021; Rucas & Alami 2022; Boiten et al. 2023). A central goal is therefore to test empirically whether one or both processes are responsible for the evolution of courtship coloration in any given system.

Intersexual selection appears to be playing a role in some taxa, where courtship coloration has been shown to be attractive to the opposite sex. Notably, these studies assess preferences within a gene pool (a single population), providing better evidence for intersexual selection than do studies of assortative mating by color across species, which can elucidate the role of courtship color in behavioral isolation (e.g. Seehausen & van Alphen 1998; Williams & Mendelson 2011). Examples of such taxa include house finches, *Carpodacus mexicanus* (Hill 1990), two-spotted gobies, *Pomatoschistus flavescens* (Amundsen & Forsgren 2001), and the butterfly *Hypolimnas bolina* (Kemp 2007). In other taxa, courtship coloration appears to influence intrasexual aggression, for example, in the red-collared widowbird *Euplectes ardens* (Pryke et al. 2002), the damselfly *Xanthagrion erythroneurum* (Khan & Herberstein 2021), and the sand lizard *Lacerta agilis* (Olsson 1994), thus potentially implicating a role for intrasexual selection in the evolution of courtship color.

In some cases, courtship color appears to function in both contexts, as a trait of "dual utility" that affects both intersexual mate choice and intrasexual aggression (Berglund et al. 1996). In the pipefish *Syngnathus typhle*, a sexually dimorphic color pattern displayed by females is attractive to males (Berglund & Rosenqvist 2001) and reduces competition from other females (Berglund & Rosenqvist 2009). In the common yellowthroat *Geothlypis trichas*, males with experimentally enlarged black masks won more fights against rival males and attracted more females (Tarof et al. 2005), and in the superb fan-throated lizard *Sarada superba,* the colorful dewlap serves as a signal both in courtship displays and in aggressive interactions between males (Zambre & Thaker 2017), although males and females prefer different dewlap colors (males prefer blue and black, females prefer orange). Although these studies suggest a dual role for nuptial coloration in an intersexual and an intrasexual context, the relative importance of nuptial coloration in courtship displays compared to territorial displays remains an open question. Here, we tested for the dual utility of courtship coloration in the rainbow darter, *Etheostoma caeruleum* (Percidae).

Darters are small, benthic, sexually dimorphic freshwater fishes native to eastern North America. Males of many species in this group display vivid color patterns during the breeding season, which appear to affect both female mate choice and male competition, at least in some species. For example, female *E. barrenense* prefer an intermediate male color (orange) over an extreme hue (red) (Williams et al. 2013), whereas female *E. spectabile* show no evidence that male coloration affects their preference (Pyron 1995; Zhou et al. 2015). Females in both *E. barrenense* and *E. zonale* strongly prefer the colors and patterns of conspecifics over those of heterospecifics (Williams & Mendelson 2011), but these are interspecific choices, and it is difficult to apply these preferences to intraspecific variation.

Courtship coloration also appears to function in intrasexual combat. Male darters are aggressive in nature, fighting over access to territories and females (Fuller 2003). Fights entail the flaring of fins, darkening in color, facing off (head-to-head), and tight circling (chasing head to tail). In the lab, contests eventually conclude with the apparent winner remaining dark in color with fins flared, while the “loser” retracts his fins and blanches in color (Sung & Mendelson unpubl. data), similar to the way copper lizards change color after losing a contest (Naretto & Chiaraviglio 2023). For intraspecific interactions, Zhou and Fuller (2016) provide evidence that coloration affects male contests in *E. spectabile*, and other studies suggest male coloration affects interspecific aggression as well. For example, the likelihood of fighting between heterospecific male darters is predicted by the similarity of their color patterns; species with more similar color patterns are more likely to fight (Martin & Mendelson 2016; Moran et al. 2017). Again, whether and how interspecific interactions apply to intraspecific interactions requires further study.

Of great interest to researchers is how and why such diverse colors and patterns evolve in darters and the role they play in speciation. Although some prior studies have focused on the role of nuptial colors and patterns either in courtship or in territorial encounters, our study evaluates the possible dual utility of the visual characteristics in both contexts. This study is an important first step towards determining which context contributes more to male color and pattern evolution in darters: courtship or territorial displays.

One complicating factor in tests of dual utility is the ability to control for the effect of interactions in one context on those in the other (e.g., the effect of male-male competition on female choice). Thus, as in Tarof et al. (2005), our experiments assessed the effect of courtship coloration in the two contexts separately, using the same males in each context. Another complicating factor for many secondary sexual traits is that they are multivariate, so it is difficult to isolate and characterize the relevant phenotypes. In the case of darters, determining the function of their color patterns is further complicated by the fact that darters have limited color vision, with only two opsin gene classes (red and green; Gumm et al. 2012). However, we focused on luminance rather than color in our study. Several studies indicate that luminance and luminance contrast are important aspects of visual signals for mate choice and sex discrimination in multiple taxa (Sztatecsny et al. 2010; Calabrese et al. 2014; Bergman et al. 2015; Cole & Endler 2015; Hawkes et al. 2019; Lynn & Cole 2019). We therefore focused on the role of luminance in intra- and intersexual interactions to ask (1) whether the most attractive males were also the best fighters, and (2) whether luminance patterns predicted either or both of these outcomes.

METHODS

Subject collection

In the spring of 2023, we collected males and females of *Etheostoma caeruleum* from streams in Maryland, USA, using a kick-seine net. We collected eight male *E. caeruleum* from Israel Creek, Frederick County (39.493097, – 77.324458) and 28 females from Bush Creek, Frederick County (39.359123, – 77.323882), Big Pipe, Carroll County (39.6139926, – 77.2390159), Furnace Branch, Montgomery County (39.2415283, – 77.4358772) and Tuscarora Creek, Frederick County (39.25193, – 77.48008). We collected the males from a site distinct from those of the females to minimize the likelihood that the focal females had previously encountered the males in this study. We identified all individuals to species based on published characteristics (Page & Burr 2011), and we confirmed breeding condition using the presence of vivid nuptial coloring in males and gravid abdomens in females. We transported all fish to the lab in aerated coolers and acclimated the subjects overnight to lab aquarium housing. Permission to collect fish was granted by the Wildlife and Heritage Service of the Maryland Department of Natural Resources (permit number SCP202246A).

Housing and care

We housed all male individuals separately in 18.9 L tanks and with opaque partitions to prevent visual contact, which could otherwise lead to aggressive behaviors and/or familiarity. We housed females together in a 151 L glass tank or individually in smaller glass tanks with visual access to one another, to avoid overcrowding. To ensure that each female was only tested once, when each female completed her trial, she was either returned to her individual housing or transferred to a new 151 L tank. As females could not observe ongoing trials and were not in physical contact with females who completed their trials, we did not expect housing condition to influence female mate preferences. Males and females were housed separately so that they did not see individuals of the opposite sex in the housing areas. Tanks were filled with tap water conditioned with Stress Coat (API) and aerated with air pumps and sponge filters. We performed weekly 10% water changes and fed the fish every other day with frozen bloodworms (as much as could be consumed in 5 min). Ambient temperature was kept at 14 °C, and the light cycle was set to mimic the natural light cycle during the breeding season (14L:10D). Each individual was assigned a unique serial number to aid identification and tracking throughout the study timeline. No individual participated in more than one trial per day, either as a focal subject or as a stimulus. Housing and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Maryland, Baltimore County (protocol 623).

VIE tagging

To distinguish between individuals during behavioral trials, we tagged all males with three brown visible implant elastomer (VIE) tags (Northwest Marine Technology) on the dorsal side of the body: one just anterior to the first dorsal fin, one between the first and second dorsal fin, and one just posterior to the second dorsal fin. We created unique tag combinations by alternating the position of the tag in each location to occur on either the left or right side, creating 2^3 (8) possible combinations. We anesthetized males in MS-222 (25 mg/mL) until the subject did not respond to touch, and gill movement was slow and steady. We placed moistened paper towels over the gills to support breathing during the procedure. We implanted all VIE tags just under the skin. Once the procedure was complete, we returned each individual to his housing and observed the subject until he resumed swimming upright. We monitored all subjects for recovery from the procedure and waited a minimum of 3 days before proceeding with experiments. We observed no adverse effects from the VIE tagging on any individuals.

Simulated territorial encounters

We performed a series of simulated territorial encounters (contests) in which all males participated in pairwise competitions for a territory. That is, the conditions in which these contests took place simulated a territorial encounter, but the contests themselves were live encounters between the males. We constructed a contest arena from a 37.9 L tank with three sides blacked out by black matte acrylic and the bottom covered with white gravel. An air pump and air stone supplied oxygen to the tank during trials. A black matte barrier divided the tank in half during acclimation. At the start of each trial, two males were acclimated to the arena, one on each side of the barrier, so that they could not see each other. After 5 min of acclimation, the barrier was removed and the males were allowed to interact freely. We observed the behavior of the males as they competed for occupation of the territory from outside the room through a window, and we recorded the trials using a Logitech webcam attached to a laptop computer. Trials were run for a minimum of 15 min and a maximum of 30 min, depending on how long it took for the contest to resolve with a clear winner and loser. We scored the trials based on previous observations of territorial competition behaviors in this species (Mendelson et al. personal obs.). A "winner" was determined when one of the males kept both dorsal fins erect, his body darkened in color, and he either occupied the middle of the arena or guarded the corner to which the losing male fled. A "loser" was determined when the male flattened his dorsal fins against his body, blanched in color, and fled to a corner of the tank, with the winning male in pursuit, and remained in the corner. If no clear winner and loser could be determined after 30 min, we declared a "draw". Each of the eight males competed against all the other males in the cohort in a pairwise fashion for a total of 28 trials. We observed no injuries or lasting physical effects in the males from these trials.

Dichotomous choice trials

Upon completion of the contest trials, we conducted dichotomous choice trials with 28 female *E. caeruleum*. Prior studies in darters show that dichotomous choice trials are effective for determining preferences in *Etheostoma* (Williams & Mendelson 2010; Martin & Mendelson 2013; O'Rourke & Mendelson 2010; Mendelson et al. 2018; Roberts & Mendelson 2020). We set up a 37.5 L focal tank with two 9.5 L tanks flush against the short sides of the focal tank (Fig. 1). We placed white gravel on the bottoms of all tanks and filled them with aquarium-conditioned water. The center of the focal tank contained brackets into which we placed clear plexiglass barriers to create an acclimation chamber. Each trial began with a 10-min acclimation period, commencing after we placed a female in the acclimation chamber and a male in each stimulus tank. For the first 5 min, matte black barriers separated the focal and stimulus tanks to block the female's view of the stimulus tanks and allow acclimation of all three fish to the experimental setup. Then the black barriers were removed, and the female could view the two stimulus males for 5 min. We then removed the clear barriers and allowed the female to move freely about the focal tank. We filmed the trials on a Logitech webcam mounted overhead the tank and recorded a minimum of 15 min of video footage per female. We tested all possible unique pairings of the same males used in the contest trials, for a total of 28 trials. We used a unique female for each trial such that no female participated in more than one trial.

We demarcated 5-cm association zones in the video recordings, one adjacent to each stimulus tank (Fig. 1), and scored the amount of time the female spent in the center zone and in each of the two association zones using the software BORIS (Friard & Gamba 2016). We used the time spent in each of the association zones to determine which male the female preferred, and to calculate her strength of preference (SOP), as SOP = (TA – TB)/(TA + TB), where TA = the time the female spent near one of the males and TB = the time she spent near his opponent (Sommer-Trembo et al. 2020).

Photography of male subjects

After behavioral trials were completed, we euthanized the males in MS-222 (250 mg/mL) and placed them in a 10% formalin solution for fixing. We used pins to position the dorsal, caudal, and anal fins such that they were fully extended. We took photographs of each male using a Canon EOS 5D Mark IV digital camera with a Canon EF 100 mm f/2.8 L macro lens attached. Fish were placed in an arena surrounded by diffuser paper and illuminated by three Canon 270EX II flashes spaced equidistant around the arena.

Analysis of visual characteristics

We used the software ImageJ (Schneider et al. 2012) to measure the standard length (tip of the nose to the caudal peduncle) of each male. Standard length was normally distributed (Shapiro-Wilk test, W = 0.8942, *P* = 0.276) rather than multimodal, suggesting males were likely within the same age cohort. Then, using the Object Selection tool in Photoshop, the background of each fish image was replaced with a plain white background. The total body area was then extracted, using the scale tool with the measured length of each male as a reference. Using custom-written scripts in Python software, RGB fish images were first converted to darter color space using known sensitivities of their visual system (Gumm et al. 2011; Hulse et al. 2020). We then extracted the mean and standard deviation of the fish luminance (over the entire body) and used those values to compute the coefficient of variation of luminance (CV), defined as the ratio of the standard deviation to the mean, commonly used to represent internal contrast in the nuptial patterns of fishes (Endler & Houde 1995; Lynn & Cole 2019; Sibeaux et al. 2019).

Elo rating calculations

We calculated Elo ratings for each male using the package EloRating in R (Neumann & Kulik 2024). Rankings were based on the outcome of the contest and choice trials separately, generating unique rankings for each context. Elo ratings were originally developed to rank chess players based on their wins, losses, and draws relative to other players and have been used in preference studies since then (Clark et al. 2018; Tribot et al. 2018; Héjja-Brichard et al. 2023). For the contest trials, we used the default value of 100 for k (a multiplier that determines the maximum number of points a competitor can gain or lose from a single contest) and the default value of 1000 for the starting value (the initial score of a new competitor entering the system). For the choice trials, we weighted the value of k based on the female strength of preference. Thus, if a female demonstrated a strong preference for a winning male compared to his opponent, that "win" would count more towards his Elo rating than a "win" in which the female demonstrated only a slight preference for one male over the other. We used the default starting value of 1000 for the choice trials.

Statistical analysis

Statistical analyses were performed using the software R (R Core Team 2024, version 4.4.1). First, we computed the Spearman correlation between Elo rankings for the contest and choice trials (n = 8 for each type of ranking) to assess the strength of the relationship between the rankings in the two contexts. Second, to determine the possible role of body area, luminance and contrast in predicting success in either context, we first assessed how those variables relate to each other by computing a Spearman correlation between all pairs, and we then used separate linear regression models, with Elo ranking (for contest or choice separately) as the dependent variable and body area, mean luminance, or contrast as the explanatory variables.

RESULTS

We found no significant correlation between Elo rankings from the simulated territorial encounters (contests) and those from the dichotomous choice trials ($n = 8$, $\rho = 0.65$, $P = 0.083$, Fig. 2). Regarding our explanatory variables, we found a significant correlation between body area and mean luminance only ($n = 8$, $\rho = -0.8571429$, $P = 0.01071$). The linear model approach suggested that Elo rankings for the intrasexual contests were not explained by any of the three variables of interest, that is, body area (n = 8, adjusted R2: 0.275, *P* = 0.1044), mean luminance (n = 8, adjusted R2: 0.2641, *P* = 0.1101), and contrast (CV, n = 8, adjusted R2: – 0.1571, *P* = 0.8307). However, contrast was a significant predictor of Elo rankings in the female choice trials (n = 8, estimate: 3777 ± 1200, R2 = 0.56, *t* = 3.148, *P* = 0.01986): the higher the CV or contrast, the higher the ranking of the male (Table 1). Similarly to intrasexual contests, female choice was not predicted by body area (adjusted R2: 0.09829, *P* = 0.2325) or mean luminance (adjusted R2: – 0.03279, *P* = 0.4118).

DISCUSSION

Our objectives were to assess the dual utility of nuptial color patterns in the rainbow darter and furthermore to identify aspects of nuptial coloration that predict success in intrasexual contests, intersexual choice, or both. First, by evaluating the same males in both contexts, in all pairwise combinations, we were able to generate a ranking (from 1-8) for each male with respect to his ability to win a territorial contest against another male and, separately, his ability to attract a female. If male phenotypes have dual utility, then the effect of those phenotypes will be aligned in these two contexts, and a male's ranking in one context should correlate with his ranking in the other. Instead, we found that the Elo rankings were not statistically significantly correlated: success in territorial contests did not correlate with attractiveness to females (Fig. 2).

This result is consistent with studies showing that females do not always prefer the most dominant males (reviewed in Qvarnström & Forsgren 1998). For example, a study on northern pintails (*Anas acuta*) showed that dominance rankings in males did not constrain female mate choice; instead, female preference may be a cause of male dominance rankings rather than a result thereof (Sorenson & Derrickson 1994). A study across eleven isogenic lines of *Drosophila melanogaster* showed no net effect of male success in dyadic territorial contests on female choice. Interestingly, in two of the lines, dominant males indeed had greater mating success; however, in one line, females preferred the "losers", suggesting that even within a species, the relationship between territorial success and mating success can vary (Cabral et al. 2008).

With respect to the dual utility of courtship coloration, our results are consistent with findings that courtship coloration may function in one context but not the other. In the house finch *Carpodacus mexicanus*, for example, females have strong association preferences for males with the most saturated red feathers, compared to yellow or orange (Hill 1990). However, saturated red coloration is not an advantage in intrasexual contests in this species; if anything, drab coloration appears to be associated with intrasexual dominance (McGraw & Hill 2000). The opposite seems true in the damselfly *Xanthagrion erythroneurum*, in which red coloration serves as a status badge for males in competitive interactions, but females do not choose mates based on color (Khan & Herberstein 2021).

For rainbow darters, we caution that our sample size, though large enough to require 56 behavioral trials across the two contexts (28 trials in each experiment), still only resulted in eight unique data points in the Elo ranking correlation analysis. This sample size may have been too small to detect a statistically significant relationship between Elo rankings. We note that the four most successful males in territorial contests (M3, M4, M5, M6) were also the four most attractive to females (Fig. 2), which is suggestive of a relationship. Of those four, M3 was the most successful in a contest and the least attractive; conversely, M5 was the most attractive and the least successful in a contest. These two males may therefore indicate that males cannot be top-ranking in both territorial contests and attractiveness; however, if these two males are outliers with idiosyncratic or unmeasured characteristics, a larger sample size may reveal a correlation between ranking in intrasexual contests and intersexual choice.

Our next objective was to determine whether any aspect of male phenotype predicted success in either or both contexts. Here, we found that success in intrasexual contests was not explained by any of our explanatory variables-body area (size), mean luminance, or luminance contrast. We did find, however, that attractiveness to females was explained by luminance contrast (CV, Table 1). Although luminance contrast between the signaler and the background has been shown to be important for mate choice (Bergman et al. 2015; Cole & Endler 2015; Lynn & Cole 2019), fewer examples demonstrate the effect of luminance contrast within signaling patterns. For example, Cole and Endler (2015) and Sibeaux et al. (2019) found no effect of within-signal luminance contrast on the attractiveness of male guppies, *Poecilia reticulata*. However, luminance contrast in wing interference patterns (WIPs) appears to be attractive to female *Drosophila simulans*. WIPs are formed by structural coloration in many insect wings, and the mean luminance and luminance contrast of male WIPs increased in experimentally evolved lineages of *D. simulans* subject to sexual selection (polyandry), but not in control lineages or in females (Hawkes et al. 2019). Males from these polyandrous lineages were also more attractive to females. Our results suggest that within-body luminance contrast in male nuptial patterns is also attractive to female rainbow darters but may not affect intrasexual contests.

Our results also found no effect of body area on Elo rankings for either intrasexual contests or intersexual choice. This was somewhat surprising, as size often matters in territorial interactions (Bee et al. 1999; García-Rosales et al. 2021; Liu et al. 2021) and in female mate choice (Côte & Hunte 1989; Wiegmann et al. 1992; Ito & Iguchi 2024); however, this is not always the case (Kimock et al. 2022). For intrasexual contests, factors such as experience can enable a competitor to overcome a size disadvantage, as for example in the territorial cichlid fish *Astotilapia burtoni* (Alcazar et al. 2014). In olive flies, *Bactrocera oleae*, prior experience in territorial contests enhanced fighting abilities in males regardless of whether prior experiences were wins or losses (Benelli et al. 2015), and in the butterfly *Pararge aegeria*, males with prior exposure to mated females became more aggressive in territorial contests and were more likely to reverse the outcome of prior contests (Bergman et al. 2010). Because we used wild-caught adult males in our study, we did not control for experience in territorial encounters or mating. Future studies could control for prior experience by rearing naïve males in the laboratory and testing the effect of experience on success in both intra- and intersexual selection contexts.

Additionally, males in some species modulate territorial and dominance-based behavior by age. In collared lizards, yearling males assume a subordinate role, while 2nd-year males engage in territorial behavior. However, in the absence of older males, yearlings are capable of displaying territorial behaviors (Baird & Timanus 1998). In the butterfly *Hypolimnus bolina*, a species in which age correlates positively with size in the spring and negatively with size in the fall, age was a better predictor of territorial contest outcomes than size (Kemp 2000). In darters, males of most species sexually mature in their first year (Page 1983; Page & Burr 2011), but reproductive activity sometimes begins or peaks in the second year (Taber & Taber 1983; Simon & Wallus 2005; Eisenhour et al. 2013). Darters continue to grow in size and gain experience throughout their lifetimes, so it is difficult to separate age from size and experience. All the males in our study had reached a size that indicates reproductive maturity (Mettee et al. 1996) and appeared to be from the same age cohort. Additional research therefore is needed to determine the effect of age and experience on success in competitive interactions in the rainbow darter.

In conclusion, we did not find support for the dual utility of nuptial color patterns in rainbow darters, as inferred from an insignificant correlation between a male's ranking in intrasexual territorial contests and his attractiveness to females. However, we note that the trend in this relationship is positive and warrants further investigation. Of the variables we measured, luminance contrast was found to predict male success, but only in attracting females. The aspects of nuptial patterns that contribute to success in male contests in this species, if any, therefore remain an open question. Our study highlights the importance of luminance contrast in mate attraction and in further evaluating the dual utility of male nuptial patterns in this species.

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DISCLOSURE STATEMENT

The authors report there are no competing interests to declare.

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ETHICAL STANDARD

Housing and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Maryland, Baltimore County (protocol 623). Permission to collect fish was granted by the Wildlife and Heritage Service of the Maryland Department of Natural Resources (permit number SCP202246A).

AUTHOR CONTRIBUTION

Conceptualization: K.M. Million, Y. Héjja-Brichard, T.C. Mendelson, M. Nasir, S. Tuckley. Methodology: K.M. Million, Y. Héjja-Brichard, T.C. Mendelson. Software: Y. Héjja-Brichard, K.M. Million. Formal analysis: K.M. Million, Y. Héjja-Brichard. Investigation: K.M. Million, Y. Héjja-Brichard, T.C. Mendelson, M. Nasir, S. Tuckley. Resources: T.C. Mendelson. Data curation: K.M. Million, Y. Héjja-Brichard, T.C. Mendelson. Writing-original draft: K.M. Million, Y. Héjja-Brichard, T.C. Mendelson. Writing-review and editing; K.M. Million, Y. Héjja-Brichard, T.C. Mendelson, M. Nasir, S. Tuckley. Visualization: K.M. Million, Y. Héjja-Brichard. Supervision: K.M. Million, Y. Héjja-Brichard, T.C. Mendelson. Project administration: K.M. Million, Y. Héjja-Brichard, T.C. Mendelson. Funding acquisition: T.C. Mendelson.

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DATA ACCESSIBILITY

Data will be made available on a public repository upon acceptance of this manuscript.

REFERENCES

Alcazar R, Hilliard AT, Becker L, Berenaba M, Fernald, RD. 2014. Brains over brawn: experience overcomes a size disadvantage in fish social hierarchies. J Exp Biol. jeb.097527. doi:10.1242/jeb.097527

Amundsen E, Forsgren T. 2001. Male mate choice selects for female coloration in a fish. Proc Natl Acad Sci USA. 98(23):13155–60. doi:10.1073/pnas.211439298

Baird TA, Timanus DK. 1998. Social inhibition of territorial behaviour in yearling male collared lizards, *Crotaphytus collaris*. Anim Behav. 56(4):989–994. doi:10.1006/anbe.1998.0865

Balmford A, Albon S, Blakeman S. 1992. Correlates of male mating success and female choice in a lek-breeding antelope. Behav Ecol. 3(2):112–123. doi:10.1093/beheco/3.2.112

Bee MA, Perrill SA, Owen PC. 1999. Size assessment in simulated territorial encounters between male green frogs (*Rana clamitans*). Behav Ecol Sociobiol. 45(3–4):177–184. doi:10.1007/s002650050551

Benelli G, Desneux N, Romano D, Conte G, Messing RH, Canale A. 2015. Contest experience enhances aggressive behaviour in a fly: when losers learn to win. Sci Rep. 5(1):9347. doi:10.1038/srep09347

Berglund A, Bizassa A, Pilastro A. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. Biol J Linn Soc. 58(4):385–399. doi:10.1006/bijl.1996.0043

Berglund A, Rosenqvist G. 2001. Male pipefish prefer ornamented females. Anim Behav. 61(2):345–350. doi:10.1006/anbe.2000.1599

Berglund A, Rosenqvist G. 2009. An intimidating ornament in a female pipefish. Behav Ecol. 20(1):54–59. doi:10.1093/beheco/arn114

Bergman M, Lessios N, Seymoure BM, Rutowski RL. 2015. Mate detection in a territorial butterfly—the effect of background and luminance contrast. Behav Ecol. 26(3):851–860. doi:10.1093/beheco/arv020

Bergman M, Olofsson M, Wiklund C. 2010. Contest outcome in a territorial butterfly: The role of motivation. Proc R Soc B. 277(1696):3027–3033. doi:10.1098/rspb.2010.0646

Boiten G, Van Iersel R, Pinxten R, Eens M. 2023. Females are more aggressive than males towards same- and opposite-sex intruders in the blue tit (*Cyanistes caeruleus*). Animals. 13(4):585. doi:10.3390/ani13040585

Bonatto F, Coda J, Gomez D, Priotto J, Steinmann A. 2013. Inter-male aggression with regard to polygynous mating system in Pampean grassland mouse, *Akodon azarae* (Cricetidae: Sigmodontinae). J Ethol. (3):223–231. doi:10.1007/s10164-013-0370-4

Bonduriansky R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol Rev. 76(3):305–339. doi:10.1017/S1464793101005693

Cabral LG, Foley BR, Nuzhdin SV. 2008. Does sex trade with violence among genotypes in *Drosophila melanogaster*? PLoS ONE. 3(4):e1986. doi:10.1371/journal.pone.0001986

Calabrese GM, Brady PC, Gruev V, Cummings ME. 2014. Polarization signaling in swordtails alters female mate preference. Proc Natl Acad Sci USA. 111(37):13397–13402. doi:10.1073/pnas.1321368111

Clark AP, Howard KL, Woods AT, Penton-Voak IS, Neumann C. 2018. Why rate when you could compare? Using the “EloChoice” package to assess pairwise comparisons of perceived physical strength. PLoS ONE. 13(1):e0190393. doi:10.1371/journal.pone.0190393

Cole GL, Endler JA. 2015. Variable environmental effects on a multicomponent sexually selected trait. Am Nat. 185(4):452–468. doi:10.1086/680022

Côte IM, Hunte W. 1989. Male and female mate choice in the redlip blenny: why bigger is better. Anim Behav. 38(1):78–88. doi:10.1016/S0003-3472(89)80067-3

Darwin C. 1871. The descent of man, and selection in relation to sex. London (UK): John Murray.

Edward DA, Chapman T. 2011. The evolution and significance of male mate choice. Trends Ecol Evol. 26(12):647–654. doi:10.1016/j.tree.2011.07.012

Eisenhour DJ, Richter AM, Eisenhour LV, Gingras C. 2013. Reproductive biology of the frecklebelly darter, *Percina stictogaster* (Teleostei: Percidae). Southeast Fish Council Proc. 54. Available from: https://trace.tennessee.edu/sfcproceedings/vol1/iss54/3/ [Accessed 10 July 2025].

Endler JA, Houde A.E. 1995. Geographic variation in female preference for male traits in *Poecilia reticulata.* Evol. 49(3):456—468. doi: 10.1111/j.1558-5646.1995.tb02278.x

Friard O, Gamba M. 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol Evol. 7(11):1325–1330. doi:10.1111/2041-210X.12584

Fuller RC. 2003. Disentangling female mate choice and male competition in the rainbow darter, *Etheostoma caeruleum*. Copeia. (1):138–148. doi:10.1643/0045-8511(2003)003[0138:DFMCAM]2.0.CO;2

García-Rosales A, Stephenson BP, Ramírez-Bautista A, Manjarrez J, Pavón NP. 2021. Female choice and male aggression in the polymorphic lizard *Sceloporus minor*. Ethology. 127(12):1053–1063. doi:10.1111/eth.13228

Gumm JM, Feller KD, Mendelson TC. 2011. Spectral characteristics of male nuptial coloration in darters (*Etheostoma*). Copeia. (2):319–326. doi:10.1643/CG-10-033

Gumm JM, Loew ER, Mendelson TC. 2012. Differences in spectral sensitivity within and among species of darters (genus *Etheostoma*). Vision Res. 55:19–23. doi:10.1016/j.visres.2011.12.005

Hawkes MF, Duffy E, Joag R, Skeats A, Radwan J, Wedell N, Sharma MD, Hosken DJ, Troscianko J. 2019. Sexual selection drives the evolution of male wing interference patterns. Proc R Soc B. 286(1903):20182850. doi:10.1098/rspb.2018.2850

Héjja-Brichard Y, Raymond M, Cuthill IC, Mendelson TC, Renoult JP. 2023. Perceptual biases, camouflage patterns, and the origin of sexual signals. bioRxiv 2023.09.27.559753. doi:10.1101/2023.09.27.559753

Hill GE. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. Anim Behav. 40:563–572. doi:10.1016/S0003-3472(05)80537-8

Hulse SV, Renoult JP, Mendelson TC. 2020. Sexual signaling pattern correlates with habitat pattern in visually ornamented fishes. Nat Commun. 11(1):2561. doi:10.1038/s41467-020-16389-0

Ito S, Iguchi K. 2024. Female mate choice for male size in a freshwater goby under experimental conditions eliminating inter-male interference. Behaviour. 161(3–4):225–246. doi:10.1163/1568539X-bja10258

Jawor JM, Young R, Ketterson ED. 2006. Females competing to reproduce: dominance matters but testosterone may not. Horm Behav. 49(3):362–368. doi:10.1016/j.yhbeh.2005.08.009

Kemp DJ. 2000. Contest behavior in territorial male butterflies: does size matter? Behav Ecol. 11(6):591–596. doi:10.1093/beheco/11.6.591

Kemp DJ. 2007. Female butterflies prefer males bearing bright iridescent ornamentation. Proc Biol Soc. 274(1613):1043–1047.

Kemp DJ, Wiklund C. 2001. Fighting without weaponry: a review of male-male contest competition in butterflies. Behav Ecol Sociobiol. 49(6):429–442. doi:10.1007/s002650100318

Khan MK, Herberstein ME. 2021. Male–male interactions select for conspicuous male coloration in damselflies. Anim Behav. 176:157–166. doi:10.1016/j.anbehav.2021.04.006

Kimock CM, Brent LJN, Dubuc C, Higham JP. 2022. Body size and canine size do not confer a competitive advantage in male rhesus macaques. Anim Behav. 187:281–290. doi:10.1016/j.anbehav.2022.01.011

Liu Z, Wickham JD, Sun J. 2021. Fighting and aggressive sound determines larger male to win male-male competition in a bark beetle. Insect Sci. 28(1):203–214. doi:10.1111/1744-7917.12748

Lynn JCB, Cole GL. 2019. The effect of against-background contrast on female preferences for a polymorphic colour sexual signal. Anim Behav. 150:1–13. doi:10.1016/j.anbehav.2019.01.023

Martin MD, Mendelson TC. 2013. Incomplete behavioural isolation and asymmetric female preference in darter sister species (Percidae: *Etheostoma*). J Fish Biol. 83:1371–1380. doi:10.1111/jfb.12239

Martin MD, Mendelson TC. 2016. Male behaviour predicts trait divergence and the evolution of reproductive isolation in darters (Percidae: *Etheostoma*). Anim Behav. 112:179–186. doi:10.1016/j.anbehav.2015.11.027

McGraw KJ, Hill GE. 2000. Carotenoid-based ornamentation and status signaling in the house finch. Behav Ecol. 11(5):520–527. doi:10.1093/beheco/11.5.520

Mendelson TC, Gumm JM, Martin MD, Ciccotto PJ. 2018. Preference for conspecifics evolves earlier in males than females in a sexually dimorphic radiation of fishes. Evolution. 72(2):337–347. doi:10.1111/evo.13406

Mettee MF, O'Neil PE, Pierson JM. 1996. Fishes of Alabama and the Mobile Basin. Birmingham (AL): Oxmoor House.

Midamegbe A, Grégoire A, Perret P, Doutrelant C. 2011. Female–female aggressiveness is influenced by female coloration in blue tits. Anim Behav. 82(2):245–253. doi:10.1016/j.anbehav.2011.04.020

Moore AJ. 1990. The evolution of sexual dimorphism by sexual selection: the separate effects of intrasexual selection and intersexual selection. Evolution. 44(2):315–331. doi:10.1111/j.1558-5646.1990.tb05201.x

Morales J, Gordo O, Lobato E, Ippi S, Martínez-de La Puente J, Tomás G, Merino S, Moreno J. 2014. Female-female competition is influenced by forehead patch expression in pied flycatcher females. Behav Ecol Sociobiol. 68(7):1195–1204. doi:10.1007/s00265-014-1730-y

Moran RL, Zhou M, Catchen JM, Fuller RC. 2017. Male and female contributions to behavioral isolation in darters as a function of genetic distance and color distance. Evolution. 71(10):2428–2444. doi:10.1111/evo.13321

Naretto S, Chiaraviglio M. 2023. Hoisting the white flag of surrender? Color change in agonistic encounters between Achala copper lizard males (*Pristidactylus achalensis*). Behav Ecol Sociobiol. 77(10):116. doi:10.1007/s00265-023-03389-5

Neumann C, Kulik L. 2024. EloRating: animal dominance hierarchies by Elo rating. R package version 0.46.18. Available from: https://github.com/gobbios/elorating [Accessed 28 December, 2025].

Olsson M. 1994. Rival recognition affects male contest behavior in sand lizards (*Lacerta agilis*). Behav Ecol Sociobiol. 35(4):249–252. doi:10.1007/BF00170705

O'Rourke CF, Mendelson TC. 2010. Male and female preference for conspecifics in a fish with male parental care (Percidae: *Catonotus*). Behav Process. 85(2):157–162. doi:10.1016/j.beproc.2010.07.005

Page LM. 1983. Handbook of darters. Neptune (NJ): TFH Publications.

Page LM, Burr BM. 2011. Peterson field guide to freshwater fishes of North America north of Mexico, 2nd ed. Boston (MA): Houghton Mifflin Harcourt.

Petrie M, Halliday T, Sanders C. 1991. Peahens prefer peacocks with elaborate trains. Anim Behav. 41(2):323–331. doi:10.1016/S0003-3472(05)80484-1

Pryke SR, Andersson S, Lawes MJ, Piper SE. 2002. Carotenoid status signaling in captive and wild red-collared widowbirds: independent effects of badge size and color. Behav Ecol. 13(5):622–631. doi: 10.1093/beheco/13.5.622

Pyron M. 1995. Mating patterns and a test for female mate choice in *Etheostoma spectabile* (Pisces, Percidae). Behav Ecol Sociobiol. 36(6):407–412. doi:10.1007/BF00177336

Qvarnström A, Forsgren E. 1998. Should females prefer dominant males? Trends Ecol Evol. 13(12):498–501. doi: 10.1016/s0169-5347(98)01513-4

R Core Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/. [Accessed 29 December 2025].

Roberts NS, Mendelson TC. 2020. Reinforcement in the banded darter *Etheostoma zonale*: the effect of sex and sympatry on preferences. Ecol Evol. 10(5):2499–2512. doi:10.1002/ece3.6076

Rosvall KA. 2008. Sexual selection on aggressiveness in females: evidence from an experimental test with tree swallows. Anim Behav. 75(5):1603–1610. doi:10.1016/j.anbehav.2007.09.038

Rosvall KA. 2011. Intrasexual competition in females: evidence for sexual selection? Behav Ecol. 22(6):1131–1140. doi:10.1093/beheco/arr106

Rucas SL, Alami S. 2022. Female–female competition occurs irrespective of patrilocality. Arch Sex Behav. 51(7):3287–3292. doi:10.1007/s10508-021-02221-3

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 9(7):671–675. doi:10.1038/nmeth.2089

Seehausen O, van Alphen JJM. 1998. The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). Behav Ecol Sociobiol. 42(1):1–8. doi:10.1007/s002650050405

Sibeaux A, Cole GL, Endler JA. 2019. The relative importance of local and global visual contrast in mate choice. Anim Behav. 154:143–159. doi:10.1016/j.anbehav.2019.06.020

Simon TP, Wallus R. 2005. Reproductive biology and early life history of fishes in the Ohio River drainage: Percidae-Perch, Pikeperch, and Darters. Vol. 4. Boca Raton (FL): CRC Press.

Sommer-Trembo C, Schreier M, Plath M. 2020. Different preference functions act in unison: mate choice and risk-taking behaviour in the Atlantic molly (*Poecilia mexicana*). J Ethol 38:215–222. doi:10.1007/s10164-020-00643-5

Sorenson LG, Derrickson SR. 1994. Sexual selection in the northern pintail (*Anas acuta*): the importance of female choice versus male–male competition in the evolution of sexually-selected traits. Behav Ecol Sociobiol. 35:389–400. doi:10.1007/BF00165841

Sztatecsny M, Strondl C, Baierl A, Ries C, Hodl W. 2010. Chin up: are the bright throats of male common frogs a condition-independent visual cue? Anim Behav. 79(4):779–786. doi:10.1016/j.anbehav.2010.01.003

Taber CA, Taber BA. 1983. Reproductive biology and age and growth of the Missouri Saddled Darter *Etheostoma tetrazonum*. Am Midl Nat. 109(2):222–229. doi:10.2307/2425401

Tarof SA, Dunn PO, Whittingham LA. 2005. Dual functions of a melanin-based ornament in the common yellowthroat. Proc Biol Sci. 272(1568):1121–1127. doi:10.1098/rspb.2005.3053

Tribot AS, Carabeux Q, Deter J, Claverie T, Villéger S, Mouquet N. 2018. Confronting species aesthetics with ecological functions in coral reef fish. Sci Rep. 8(1):11733. doi:10.1038/s41598-018-29637-7

Weiss SL, Dubin M. 2018. Male mate choice as differential investment in contest competition is affected by female ornament expression. Curr Zool. 64(3):335–344. doi:10.1093/cz/zoy023

Wiegmann DD, Baylis JR, Hoff MH. 1992. Sexual selection and fitness variation in a population of smallmouth bass, *Micropterus dolomieui* (Pisces: Centrarchidae). Evolution. 46(6):1740–1753. doi:10.2307/2410028

Williams TH, Mendelson TC. 2011. Female preference for male coloration may explain behavioral isolation in sympatric darters. Anim Behav. 82(4):683–689. doi:10.1016/j.anbehav.2011.06.023

Williams TH, Mendelson TC. 2010. Behavioral isolation based on visual signals in a sympatric pair of darter species. Ethology. 116(11):1038–1049. doi:10.1111/j.1439-0310.2010.01816.x

Williams TH, Gumm JM, Mendelson TC. 2013. Sexual selection acting on a speciation trait in darters (Percidae: *Etheostoma*). Behav Ecol. 24(6):1407–1414. doi:10.1093/beheco/art080

Zambre AM, Thaker M. 2017. Flamboyant sexual signals: multiple messages for multiple receivers. Anim Behav. 127:197–203. doi:10.1016/j.anbehav.2017.03.021

Zhou M, Fuller RC. 2016. Intrasexual competition underlies sexual selection on male breeding coloration in the orangethroat darter, *Etheostoma spectabile*. Ecol Evol. 6(11):3513–3522. doi:10.1002/ece3.2136

Zhou M, Loew ER, Fuller RC. 2015. Sexually asymmetric colour-based species discrimination in orangethroat darters. Anim Behav. 106:171–179. doi:10.1016/j.anbehav.2015.05.016

TABLES

Table 1.

Output of a linear model predicting Elo rankings for female strength of preference as a function of CV. CV corresponds to the coefficient of variation of luminance and is defined as the ratio of the standard deviation to the mean.

Parameters	Estimate	SE	t-value	*P*-value
Intercept	587	132	4.446	**0.00435**
CV	3777	1200	3.148	**0.01986**

Residual SE: 42.46 on 6 degrees of freedom
Multiple R2: 0.6229, Adjusted R2: 0.56
F statistic: 9.91 on 1 and 6 df, *P*-value: 0.01986

FIGURES

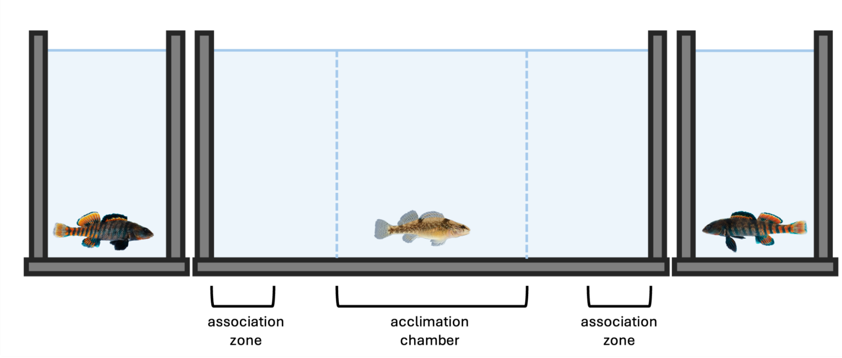

Fig. 1. — Dichotomous mate preference paradigm. The main measure is the time that the focal fish (in the center) spends in either association zone adjacent to either individual of the opposite sex.

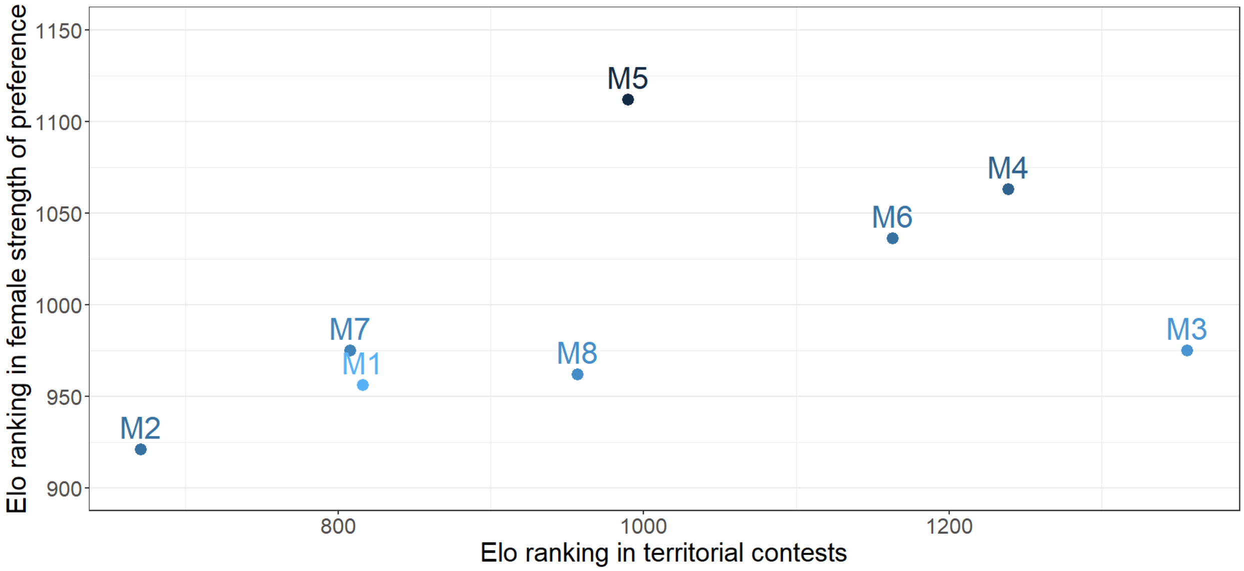

Fig. 2. — Elo rankings for the dichotomous choice trials plotted against Elo rankings for the simulated territorial encounters. The darkness of the dot indicates the value of the coefficient of variation of luminance (CV) for that individual: the darker the color, the higher the CV.