Original Article

The evolutionary loss of a badge of status alters male competition in three-spine stickleback

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The evolutionary loss of sexual signals is taxonomically widespread and quite common. These signals are often important not only in mate choice but also in male competition for territories and females; yet, male competition has rarely been investigated in the context of signal loss. We asked whether the loss of red throat color in three-spine stickleback (*Gasterosteus aculeatus*), an ancestral signal important in both male competition and mate choice, is accompanied by changes to intrasexual selection. Several freshwater stickleback populations have lost the red throat, and instead develop black breeding coloration. Previous work demonstrated that sensory drive is likely responsible. We conducted trials that mimic contact between ancestral (red) and derived (black) types during territory and nest establishment under conditions mimicking the 2 water color environments (clear and red-shifted, respectively) where they are found. We found that the allocation of competition behaviors depends on male breeding color, but not water color or the interaction of the 2. Although the total number of aggressive behaviors did not vary between color types, black males directed more aggression toward red males. Assessing each male competition behavior separately revealed that this pattern was largely driven by black fish biasing "charges" toward opposite type males. Agonistic behavior between types may strengthen divergence in resource or habitat use between populations of males with and without the ancestral sexual signal.

Key words: **female choice, male competition, sexual signal, three-spine stickleback, trait loss**.

INTRODUCTION

Trait simplification, loss, and reversal are widespread and contribute broadly to patterns of extant diversity across taxa (Lahti et al. 2009). Many studies show the loss of traits to be at least as common, if not more so, than the evolution of novel traits (e.g., Wiens 2001; Porter and Crandall 2003); notable examples include the dozens of independent losses of limbs in tetrapods (Wiens et al. 2006), the repeated loss of sight in cave-dwelling organisms (Niven 2008), and the repeated loss of body armor in sticklebacks invading freshwater (Colosimo et al. 2004). The loss and reduction of sexually selected signals has received less attention. Although research on sexual selection emphasizes directional selection for exaggerated male traits and strong female preferences (Kirkpatrick and Ryan 1991; Andersson 1994; Mead and Arnold 2004), studies in a diverse array of taxa ranging from insects to vertebrates show that attractive male traits are frequently lost or reduced over evolutionary time (Reimchen 1989; Burns 1998; Wiens 1999; Scott 2001; Morris et al. 2005; Wong and Rosenthal 2006; Zuk et al. 2006; Ptacek et al. 2011; Kang et al. 2013; reviewed in Wiens 2001). Like

other trait types, sexual signal losses frequently outnumber gains (Andersen 1997; Burns 1998; Wiens 1999; Kang et al. 2013), making this an important contributor to diversity in signaling systems.

The loss of exaggerated male signals implies that other forces (natural selection, drift, or some combination of the 2) can overtake sexual selection, or sexual selection becomes weaker or reverses direction over time (Wiens 2001). There is growing support for the idea that female preferences are often lost or reversed in groups where male signals have been lost (McClintock and Uetz 1996; Saetre et al. 1997; Basolo 1998; Morris 1998; Morris et al. 2005; Wong and Rosenthal 2006; Ptacek et al. 2011). Sexual signals, however, frequently play a role in both intersexual and intrasexual selection as mate attractants and badges of status (Berglund et al. 1996). Male–male interactions that depend on conspicuous signals should be affected by changes to those signals just as mate choice is; yet, impacts of signal loss on intrasexual selection have largely been ignored.

To our knowledge, male competition has only been assessed in the context of the loss of sexual signals in 1 system, field crickets (*Teleogryllus oceanicus*) that have lost the ability to sing (Logue et al. 2010). Interactions between males who cannot sing are characterized by greater aggression than interactions involving 1 or 2

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males who can sing (Logue et al. 2010). The loss of song, then, disrupts the crickets' ability to alleviate the costs of fighting via displays. Additionally, in 3 groups undergoing ecological speciation, Lake Victoria cichlids (Seehausen and Schluter 2004), lizards at White Sands (Robertson and Rosenblum 2010), and limnetic and benthic three-spine sticklebacks in British Columbia (Lackey and Boughman 2013), both male competition and mate choice have been influenced by changes in the habitat that alter signal detectability. In these groups, competition between males promotes diversification. For instance, in Lake Victoria cichlids, males direct more aggression toward the most common color morph during competition for breeding sites, and rare color morphs enjoy a fitness advantage, promoting color diversification (Seehausen and Schluter 2004).

Here, we address 2 important open questions 1) does the loss of a sexual signal alter intrasexual competition behavior and 2) do changes to the signaling environment (perhaps responsible for signal loss) mediate responses to individuals with and without the ancestral signal? Our study system is freshwater populations of three-spine sticklebacks, *Gasterosteus aculeatus* from Washington State. In the breeding season, most male three-spine sticklebacks, including the marine ancestors of freshwater populations, develop a red throat that extends from the mouth to the pelvic spines, and contrasts with a blue back and eye (Baube et al. 1995). In several locations along the Pacific coast of North America, males have lost the ancestral red throat and instead are drab during courtship and mate choice and turn increasingly black through the breeding season and into the parental phase (McPhail 1969; Semler 1971; Moodie 1972; Reimchen 1989; Scott 2001; Scott 2004; Supplementary Figure S1). For simplicity, we will refer to these as black sticklebacks, as McPhail (1969) did. Color differences have a genetic basis (McPhail 1969; Hagen and Moodie 1979; Malek et al. 2012). In locations where black sticklebacks are found, the water is often red shifted in color due to increased tannins (the Queen Charlotte Islands, Reimchen 1989; Enos Lake British Columbia, Boughman 2001; Washington, Scott 2001), which reduces the visibility of red signals (Boughman 2001; Scott 2001; Scott 2004). The switch to black coloration is consistent with the sensory drive hypothesis, which predicts that the evolution of signals may shift to improve transmission in a new environment (Ryan 1990; Ryan et al. 1990; Endler 1992; Boughman 2002). In parallel with work on signal loss, sensory drive research also focuses on female mate choice, and largely ignores male competition (Robertson and Rosenblum 2010; Maan and Seehausen 2011).

Female preference for red throat color is well established in three-spine sticklebacks, including in ancestral marine groups (Ter Pelwijk and Tinbergen 1937; Milinski and Bakker 1990, 1992; Bakker and Milinski 1991; Baube et al. 1995; McKinnon 1995; Rowland et al. 1995; Tinghitella et al. 2013). In addition to its mate choice role, red throat coloration is important in male competition for territories (e.g., Bakker and Sevenster 1983). Territory acquisition and defense directly influence male fitness because males without nests cannot accept eggs, and aspects of the nest and site influence female choice (Barber et al. 2001; Ostlund-Nilsson and Holmlund 2003). In early investigations, brighter red male three-spine sticklebacks appeared to be attacked more frequently and intensively (Ter Pelwijk and Tinbergen 1937; Tinbergen 1948) than dull males. More recent work suggests instead that red males receive fewer attacks than dull males (Rowland 1982; Rowland 1994; Rowland et al. 1995; Baube 1997), suggesting the red signal is a "threat" that mitigates the cost of agonistic interactions (Maynard-Smith and Harper 2003).

The role of red throat coloration in male competition suggests that its loss in south west Washington populations may be accompanied by changes in intrasexual selection. We simulated competition for territories and nest establishment by housing fish from red and black populations in seminatural breeding conditions where multiple males and multiple females interact, under lighting conditions that mimic the clear and red-shifted water color where they are found in the field. We made the following predictions. If changes in male signals are independent of changes in male competition, and signals mitigate the cost of agonistic interactions (Maynard-Smith and Harper 2003), we expected males from red and black populations to direct fewer competitive behaviors toward red males, particularly in full-spectrum lighting. If, however, changes in male competition accompany the loss of this sexual signal, we expected for 1 morph to be more aggressive (e.g., Mikami et al. 2004; Dijkstra et al. 2010) or for males to be more aggressive to phenotypically similar individuals (e.g., Seehausen and Schluter 2004; Dijkstra et al. 2007; Lehtonen 2014). Finally, if changes to the sensory environment mask the sexual signal, facilitating the loss of red coloration, we expected competition with red males to be reduced in red-shifted environments where they are less visible.

METHODS

Three-spine sticklebacks were collected from south west Washington, US rivers and streams. Red fish were collected from Wishkah River (47°0′17″N, 123°48′49″W, $\mathcal{N}$ = 24), Satsop River (46°59′59″N, 123°29′38″W, $\mathcal{N}$ = 25), Chehalis River (46°56′22″N, 123°18′46″W, $\mathcal{N}$ = 48), and Campbell Slough (47°2′40″N, 124°3′33″ W, $\mathcal{N}$ = 21). Black fish were collected from Vance Creek (46°59′48″N, 123°24′43″W, $\mathcal{N}$ = 19), Scatter Creek (46°49′20″N, 123°3′11″W, $\mathcal{N}$ = 47), Conner Creek (47°4′10″N, 124°9′58″W, $\mathcal{N}$ = 70), and Black River (46°49′45″N, 123°8′1″W, $\mathcal{N}$ = 29) for a variety of studies (Supplementary Figure S2). Fish were transferred to the University of Denver, separated by sex, and housed with others from the same location in visually isolated 110-L tanks (77 × 32 × 48 cm) at a density of approximately 10–20 fish per tank. Tanks were housed in a temperature and photoperiod controlled room set to 17 °C and 15:9-h light:dark at the beginning of the experiment. Temperature and photoperiod conditions tracked those occurring in south west Washington to simulate breeding conditions throughout the season. Fish were fed bloodworms and brine shrimp daily. Lights in the room are broad-spectrum Sylvania Octron Eco 5000-K fluorescent lights and the light in the 110-L holding tanks was not red shifted in color.

To assess differences in the phenotypes of black and red fish, we measured the color and length of all individuals used in competition trials. Color measurements were taken by eye while fish were in the competition trial tanks using a standardized color scoring method (Boughman 2001, 2007; Lewandowski and Boughman 2008; Lackey and Boughman 2013; Tinghitella et al. 2013) that closely matches reflectance data (Albert et al. 2007; Boughman 2007). Color measurements were taken twice per replicate, once each in full-spectrum and red-shifted light, following their respective 24-h acclimation periods (see below). This allowed us to assess plasticity in color across sensory environments. For each male, we recorded the area expressing red, the intensity of red, and overall body darkness. We scored each color component on a scale from 0 to 5. A score of 0 represented no color and a score of 5 represented maximum color or intensity. To measure fish length, we photographed individuals at the completion of each replicate using

a Canon Powershot G15 under standardized lighting in a dark box with a metric ruler for scale. The photo process takes less than 30 seconds. Fish were then immediately placed into 110-L holding tanks. Photos were imported into TPSDIG2 (Rohlf 2006a, 2006b). To determine standard length, the distance between digital landmarks at 2 extremes of the fish (the anterior tip of upper lip and caudal border of the hypural plate at the lateral midline) was calculated according to established methods (Taylor et al. 2006; Cooper et al. 2011).

Competition trials were conducted in 284-L tanks (123 × 47 × 54 cm) with a gravel bottom, artificial plants, and flower pots for cover. Three males and 3 females from a red population and 3 males and 3 females from a black population were chosen at random and placed in a tank (12 fish per replicate). Prior to replicate assignment, all fish were marked with colored elastomer tags (Northwest Marine Technology Inc.) so that we could identify their color type and sex. The design mimicked competition following the establishment of black fish in ancestrally red populations, or, alternatively, in current regions of sympatry. In each replicate, males and females of a given coloration were from the same population. Population combinations were never repeated and included (red × black): Satsop × Scatter, Satsop × Vance, Wishkah × Black River, Wishkah × Conner, Wishkah × Scatter, Chehalis × Black River, Chehalis × Conner, Chehalis × Scatter, Chehalis × Vance, Campbell × Black River, Campbell × Conner, and Campbell × Scatter. There were 2 separate sets of 3 Satsop, Chehalis, Scatter, and Black River males. A given set of 3 males was used 1–3 times, but always with a different set of females of the same color type and a different population of opposite color fish. In 3 replicates, fewer than 3 males were available from 1 population, so we used a reduced number of males and matched the number used across the 2 color types.

To determine whether or not competitive interactions depend on the sensory environment, we conducted trials under full-spectrum and red-shifted light conditions. A replicate consisted of 1 trial in full-spectrum light and 1 in red-shifted light, conducted in random order. We ran 12 replicates. We allowed 24 h for acclimation to the sensory environment before the first trial began and an additional 24 h when the lighting conditions were changed. In aquatic systems, objects tend to be illuminated from above by broad-spectrum downwelling light. Dissolved organic matter in the water attenuates light at the blue end of the visible spectrum causing red-shifted horizontal light, on which objects are viewed from the side (Endler 1983). To produce the red-shifted sensory environment in the lab, we placed a colored theatrical gel that eliminated wavelengths below 480 nm (Rosco E-Color 15 Deep Straw) around the tank, such that conspecifics were viewed against a background of red-shifted horizontal light (Figure 1). We also standardized illumination (Supplementary Appendix S1). This gel was chosen to match the spectral distribution of ambient light in natural red-shifted habitats (Boughman 2001; Scott 2001; Lewandowski and Boughman 2008), including those in south west Washington (Scott 2001).

Each trial lasted 1 h and took place between 1000 and 1400 h. During a trial, 3 observers recorded all behaviors indicating male–male competition in real time using the event recorder JWatcher (Blumstein et al. 2006; Supplementary Table S1). One observer recorded behaviors initiated by and received by the black fish, the second recorded behaviors initiated by and received by the red fish, and the third provided confirmation that interacting fish were identified correctly by sex and color type. We observed 85.43 ± 11.25 male competition behaviors per trial. Behaviors indicating female
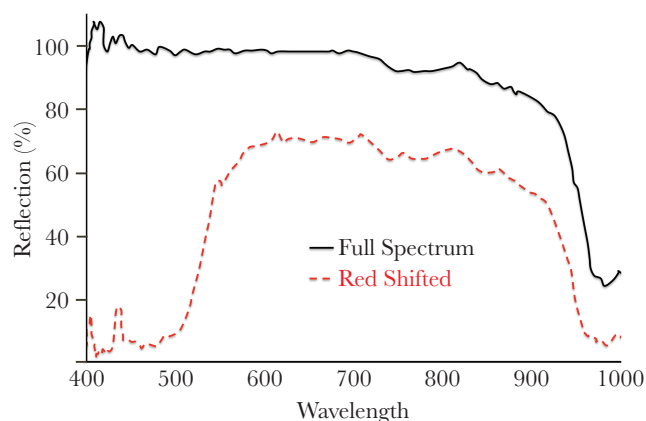

Figure 1
Reflection of light with (red-shifted) and without (full-spectrum) theatrical gel recorded underwater with an Ocean Optics JAZ spectrophotometer, 600-2-UV/VIS optical fiber, and CC-3-UV cosine corrector.

preference (directed by females toward males), male courtship (directed by males toward females), and female–female approaches were also observed during competition trials (Supplementary Table S1). We recorded them and report them below, but caution that intersexual interactions represent only early stages of courtship because males did not have complete nests and females were a mix of gravid and not gravid. Furthermore, the experimental design does not allow us to disentangle female preferences from male competition. Preferences expressed may have been influenced by which males dominated access to females. In short, we did not expect for female–female or male courtship behaviors to vary between red and black populations because females do not express nuptial coloration in these populations and male choice is typically for large females (Kraak and Bakker 1998). Previous work in these populations (McPhail 1969; McKinnon 1995) suggests females retain ancestral preferences (but see Scott 2004), so we expected no difference in preferences between females from red and black populations, and for both types to prefer red males.

Statistical analyses

We compared the lengths of red and black sticklebacks using *t*-tests for each sex. To determine whether male color is plastic depending on sensory environments, we used restricted maximum likelihood mixed models with fish color type (red vs. black), sensory environment (full-spectrum or red-shifted), and their interaction as fixed effects, and replicate as a random effect. The response variables in color models were the red area, red intensity, and body darkness measures described above. Each trait was assessed in a separate model. All analyses were performed in JMP version 11.0.

We then used 4 REML mixed models to assess differences in fish behavior between color types and sensory environments. We ran 1 model for each type of behavioral interaction assessed (male competition, female–female, female preference, and male courtship). Fixed factors in the models included fish color type, sensory environment, and their interaction. We included both replicate and the identities of the acting fish as random effects (Supplementary Appendix S1), and the overall number of behaviors (of that type) observed was a covariate. The dependent variable was the difference in behaviors directed toward red versus black fish. The differences in female preference and male courtship behaviors directed toward red versus black fish were square root transformed to

improve normality, but transformation was not required for male competition or female–female behaviors. Because body length differs between red and black fish (see Results for details), we also included the difference in length of red and black males as a covariate in the male competition and female preference models, and the difference in length of red and black females as a covariate in the male courtship and female–female models. Running the models without the size difference covariate produced qualitatively equivalent results.

Finally, to address whether 1 color type was more aggressive in competition, we used 2 REML mixed models to compare the number of overall competition behaviors and the number of more aggressive competition behaviors (bites + chases) between color types (Supplementary Appendix S1). Focal fish identity was a random factor in the model, which also included the difference in length of red and black males as a covariate. All means reported are least squares (LS) means ± 1 standard error.

RESULTS

Phenotypic differences between red and black fish

As expected, throat and body coloration differed markedly between male types. Red males had greater throat area and intensity than black males, whereas black males had darker overall body color (Table 1). We found no evidence for plasticity in color across sensory environments on the timescale assessed, or for interactions between male color type and sensory environment on aspects of male color (Table 1; but see Lewandowski and Boughman 2008). Additionally, red and black fish differed in length. Red males were 50.68 ± 0.64 mm and black males were 48.16 ± 0.76 mm ($t = 2.54$, $P = 0.017$, $N = 33$). Females from red locations were 49.77 ± 1.06 mm and females from black locations were 43.36 ± 0.76 mm ($t = 4.92$, $P < 0.001$, $N = 27$).

Intrasexual selection: male competition and female–female interactions

The allocation of competition behaviors toward red and black males depended on color type of the acting male, but not on sensory environment or their interaction (Table 2). Overall, black males biased their aggression (charges + bites + chases) toward red males (Figure 2). Red males directed competition behaviors toward the 2 color types approximately equally (95% confidence intervals [CIs] include 0; Figure 2).

We found no evidence for increased intensity of aggression by 1 type of male when competition behaviors were assessed overall or assessed by intensity of the behavior. The sum number of male competition behaviors performed by black and red males did not differ (red: 52.43 ± 10.57, black: 32.43 ± 10.72; $F_{1,\ 9.5} = 1.77$, $P = 0.214$), nor did the number of more aggressive behaviors (bites + chases) (red: 3.35 ± 2.49, black: 5.09 ± 2.52; $F_{1,\ 8.1} = 0.24$, $P = 0.636$).

To learn more about how specific behaviors were allocated by red and black males, we assessed charges, bites, and chases in separate REML mixed models. Male color type was a fixed factor, fish size and number of behaviors were covariates, and focal fish identity was a random factor. The response variables were the difference in the number of charges, bites, and chases, respectively, directed to red versus black males. We found that the difference in male competition behavior between male types is largely due to the allocation of charges, by far the most commonly observed competition behavior. Black males tended to allocate bites and chases toward red competitors also, but the differences were not significant (both $P > 0.06$). During charges, a focal male swims very quickly toward an opponent; these often occur just prior to a bite or chase. The allocation of charges did depend on color type of the acting male ($F_{1,\ 8.7} = 30.22$, $P < 0.001$). Black males directed 20.11 ± 2.79 more charges toward red than black males (95% CI do not include 0), and red males exhibited no competitor preferences, allocating 1.78 ± 2.74 fewer charges toward black than red males (95% CI do include 0).

In contrast, female–female interactions did not depend on the color type of the acting individual, the sensory environment, or their interaction (Table 2). Female–female interactions consisted of only approaches. The difference in behaviors directed toward red versus black females by red females was -1.38 ± 1.26 in full-spectrum light and -1.56 ± 1.14 in red-shifted light. The difference in behaviors directed toward red versus black females by black females was -0.62 ± 1.19 in full-spectrum light and 3.08 ± 1.19 in red-shifted light.

Intersexual selection: female preferences and male courtship

Females from red versus black populations allocated their interest behaviors toward males with and without the red throat similarly (Table 2, Figure 3a); both types prefer red to black males (95% CIs do not include 0; Figure 3a). The allocation of male courtship behaviors to females from red versus black populations was also

Table 1

Results of REML mixed models examining the effects of fish color type, SE, and their interaction on male red throat area, red throat intensity, and body darkness

Color trait	Color type	Mean ± standard error	Fixed effect	F	df	P
Throat area	Red	0.68 ± 0.21	Color type	**11.44**	**1,57.0**	**0.001**
	Black	0.00 ± 0.00	SE	0.73	1,53.3	0.397
			Color type × SE	0.73	1,53.3	0.397
Throat intensity	Red	0.47 ± 0.13	Color type	**14.50**	**1,58.0**	**<0.001**
	Black	0.00 ± 0.00	SE	0.21	1,53.8	0.649
			Color type × SE	0.21	1,53.8	0.649
Darkness	Red	1.16 ± 0.11	Color type	**20.47**	**1,60.1**	**<0.001**
	Black	2.23 ± 0.15	SE	0.45	1,54.2	0.507
			Color type × SE	0.21	1,54.2	0.650

SE, sensory environment.
Significant P-values are in bold. Each unique male used in trials was included only once in the color models.

Table 2

Results of REML mixed models examining factors that influence the difference in number of behaviors directed toward red versus black fish in male–male competition, female–female interactions, female preference, and male courtship

Behavior type	Fixed effect	F	df	P
Male competition	Color type	**25.55**	**1,9.6**	**<0.001**
	SE	0.001	1,34.3	0.974
	Color type × SE	0.001	1,34.2	0.979
Female–female	Color type	5.06	1,4.8	0.077
	SE	2.16	1,28.4	0.152
	Color type × SE	3.30	1,23.6	0.082
Female preference	Color type	1.01	1,3.3	0.383
	SE	0.03	1,14.9	0.858
	Color type × SE	1.37	1,14.8	0.260
Male courtship	Color type	1.50	1,7.1	0.260
	SE	0.09	1,14.6	0.766
	Color type × SE	0.49	1,13.9	0.497

SE, sensory environment.
Significant P-values are in bold.
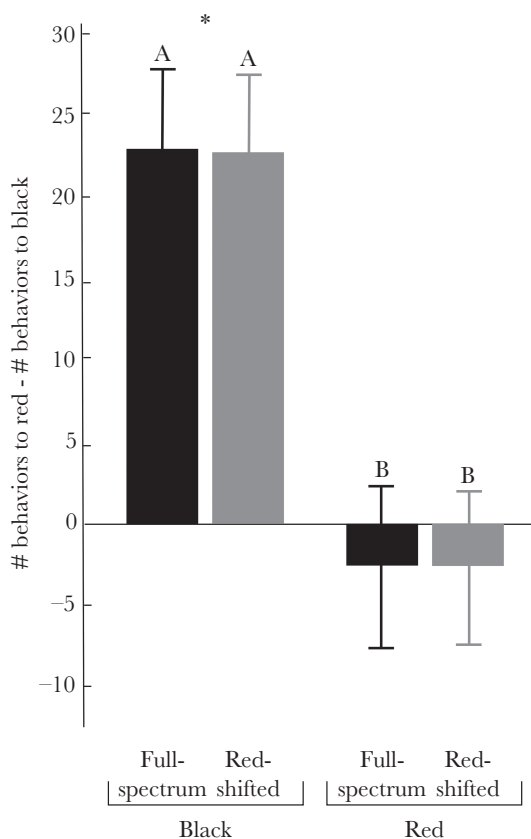
Figure 2
Difference in intrasexual competition behaviors directed toward red versus black males is given across male color types and sensory environments. Pictured are LS means ± 1 standard error. Letters above the bars indicate significant differences in a post-hoc Tukey's test (α = 0.05). *Indicates 95% CIs for color type LS means do not include 0.

independent of male color (Table 2, Figure 3b). Finally, we found no evidence that the allocation of courtship or preference behaviors differs across sensory environments or varies with the interaction of fish color and sensory environment (Table 2, Figure 3).

DISCUSSION

We used competition trials that mimicked the establishment of territories and nests to determine whether sexual signal loss is associated with changes in intrasexual competition and whether variation in the sensory environment mediates those interactions. We found that male three-spine sticklebacks lacking the red throat signal adjusted their competitive responses to others depending on their nuptial color phenotypes, but red males did not, suggesting the potential for rapid evolution of intrasexual interactions (Table 2, Figure 2). In line with recent research, we predicted that if differences in aggression existed between morphs, either red or black males would be more aggressive, or aggression would be directed toward phenotypically similar individuals. Instead, we found that black males directed more competition (largely charges) toward red males, whereas red males attacked the 2 types equally (Figure 2). We found no evidence for variation in behavior across sensory environments, perhaps because we only eliminated horizontal full-spectrum light. Female–female interactions provide a nice comparison—they do not depend on color type, sensory environment, or their interaction, as expected, given that females do not express nuptial coloration in these populations.

The aggression patterns outlined suggest either increased aggression toward red males in black populations, or decreased aggression toward red males in red populations. It has been hypothesized that red throat coloration honestly indicates fighting ability and mitigates the costs of fighting. In black populations where red throats are uncommon and difficult to see (Boughman 2001), selection to avoid competition with these (high-quality) males may be relaxed. If this is the case, that black sticklebacks direct more aggression toward red males is consistent with aspects of Logue et al's. (2010) study of aggressive encounters between silent and calling male field crickets. In that study, encounters involving at least 1 silent male were more aggressive because, without song, males could not mitigate the costs of fighting (Logue et al. 2010). In the sticklebacks, if red signals fighting ability and relaxed selection in black populations means males no longer avoid competing with bright red males, the loss of the signal similarly prevents males from mitigating the costs of fighting via signals. Additionally, if further studies confirm that females prefer red over black males, the male competition pattern observed makes adaptive sense—black males would do better in mate choice if their neighbors were also black.

The pattern of increased aggression toward opposite type males differs from much previous work on aggression biases (Qvarnstrom et al. 2012). Several recent investigations show instead that aggression is biased toward similar competitors (e.g., Dijkstra et al 2007; Pauers et al. 2008; Lehtonen 2014), presumably because males recognize them as more likely to share
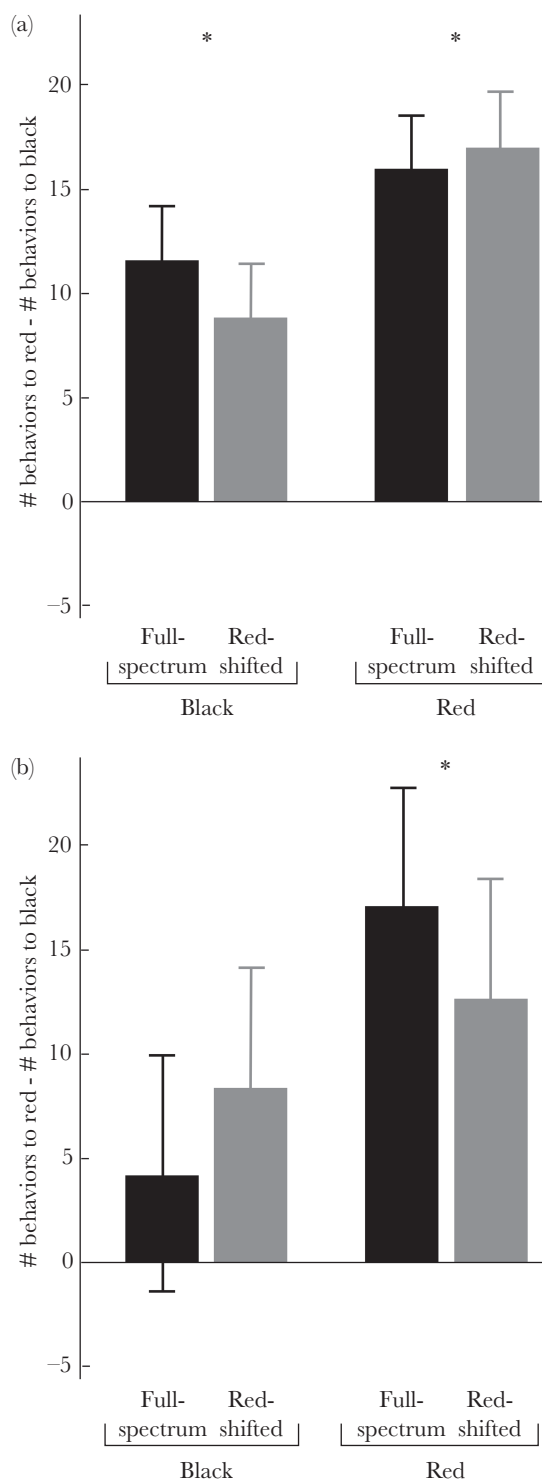
Figure 3

The difference in intersexual mate choice behaviors directed toward red versus black fish is given across color types and sensory environments. Female courtship interest behaviors directed toward males are depicted in (a) and male courtship behaviors directed toward females are depicted in (b). Pictured are LS means ± 1 standard error. * Indicates 95% CIs for color type LS means do not include 0.

important limiting resources (Seehausen and Schluter 2004; Grether et al. 2009). Increased aggression toward similar males drives frequency-dependent selection, which can promote the

invasion of new traits and the coexistence of multiple morphs (Qvarnstrom et al. 2012). Agonistic behavior between types, as observed here, however, is instead thought to select for divergence in resource or habitat use (Adams 2004; Peiman and Robinson 2007).

We predicted that competition with red males would be reduced in red-shifted environments where these signals are less visible, but found no differences in the allocation of competition (or other behaviors) across sensory environments (Table 2, Figures 2 and 3). The lack of an effect of sensory environment on male competition suggests that water color does not mediate competition between red and black males. Our results are inconsistent with work by Baube (1997), who found that changes in the lighting environment that mask red signals eliminate the dominance effect of the red throat in three-spine sticklebacks from Long Island, NY. This may be because Baube (1997) eliminated all full-spectrum light and we shifted only the horizontal light.

Although we hesitate to conclude much about mate choice from these trials, our female choice results are consistent with findings in swordtails and Pacific field crickets that have lost a sexual signal, in that preferences of female sticklebacks from black populations do not appear to differ from those of red populations, as predicted (Morris et al. 2005; Tinghitella and Zuk 2009; Figure 3a, Table 2). This pattern holds regardless of sensory environment. In addition to obvious differences in throat color, body color, and darkness (Table 1), black fish of both sexes are smaller than red fish. This suggests the evolution of body size differences in addition to color differences. In this experiment, then, we cannot distinguish between a preference for red over black and a preference for large body size (or 1 for red coloration that is masked by body size). Moreover, the absence of an effect of light treatment bolsters the interpretation that females prefer large males in these populations. However, although the difference in male length between red and black populations is statistically significant, it is also minimal (just more than 2 mm difference in length). A previous study (McKinnon 1995) in which a videotaped courting male was manipulated to have red or black color, also suggests that females from North American populations of red and black sticklebacks do not differ in color preferences, so perhaps both color and size are important. Our female preference findings are inconsistent with 1 study of red and black Washington populations (Scott 2004), and variation in experimental design may be key. Scott (2004) found that red and black types mate assortatively in a design that assessed female choice in the absence of male competition. Unambiguous evidence for the presence or absence of changes in female preferences would come from in situ mate choice experiments, with size-matched males, which we plan to conduct.

Finally, like female preferences, the allocation of male courtship behavior appears not to have changed with the loss of the red throat, as predicted (Figure 3b, Table 2). Male mate choice for larger females is strong in some populations of sticklebacks because males make a substantial reproductive investment (Kraak and Bakker 1998). We interpret the preferential courtship of females from red populations to mean that males of both color types prefer large mates.

In summary, the loss of sexual signals may impact both intersexual and intrasexual selection. Future work should focus on how divergence in male competition, like the pattern of increased aggression to different type males, contributes to reproductive isolation.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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