

Mate choice based on complex visual signals in the brook stickleback, *Culaea inconstans*

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In species with complex courtship, females often have the opportunity to assess multiple male cues during mate choice. Depending on the strength and form of the coevolutionary relationships between male traits and female preferences, geographic variation in signal complexes can result in a variety of mate choice scenarios ranging from partial to complete positive assortative mating. Using mate choice trials, we investigated the relationship between intrapopulation female biases for complex visual signals and interpopulation mating dynamics in 2 populations of brook stickleback, *Culaea inconstans*, from distinct mitochondrial DNA genetic lineages. Our results showed that courtship effort is the primary visual criterion of mate assessment in this species; females from both lineages exhibited directional preferences for increased behavioral vigor. In contrast, male nuptial color intensity and body size had little influence on female choice decisions. Interpopulation divergence in male traits was not strongly correlated with divergence in female preferences, producing asymmetric patterns of assortative mate choice across the lineage divide. We compare female preferences for male visual traits in *C. inconstans* with other members of the Gasterosteidae and suggest that mate assessment differs substantially between stickleback species. *Key words*: assortative mating *Gasterosteus*, color, courtship, female preference, reproductive isolation, speciation, visual signals. [*Behav Ecol*]

In most sexually reproducing species with active mate choice, signaling and receiver systems are highly complex entities; male cues are often hierarchical in nature, consisting of a variety of traits for female assessment within each sensory modality (e.g., visual signals such as size, behavior, color), each one of which may in turn be composed of several different elements (e.g., the hue, intensity, or pattern of color). In addition, both within and among populations, females can vary in their response to both single male traits and multicomponent signals in complicated ways (Blows et al. 2003; Rundle et al. 2008).

Between populations, geographic variation in trait–preference complexes can result in a variety of mate choice scenarios ranging from partial, or asymmetric, to complete positive assortative mating (Lande 1981; West-Eberhard 1983; Ptacek 2000; Panhuis et al. 2001; Turelli et al. 2001). For example, if interpopulation divergence in female preferences closely tracks divergence in male traits, then genetically covarying signal–preference complexes can lead to behavioral isolation and, ultimately, speciation (e.g., Boul et al. 2007; see also references in Foster and Endler 1999). However, reproductive isolation between groups may be constrained if mean female preference values and male signals fail to coevolve in concert (Schwartz and Hendry 2006), the specific outcome of which will depend on the strength and form of the trait–preference relationship. For example, open-ended directional female biases for male traits that are mismatched with respect to mean male population expression values (e.g., Hill 1994; Houde and Hanks 1997; Hamilton and Poulin 1999) can lead to asymmetrical patterns of interpopulation mate choice favoring males from populations who exhibit the highest trait means.

One of the most valuable ways to obtain information about how the form and shape of trait–preference complexes within

populations (e.g., Schluter 1988; Ritchie 1996) influences mating dynamics among groups is to examine divergence within taxa that have undergone the initial stages of speciation (Schwartz and Hendry 2006). Here, we compare patterns of female response to multiple male visual signals within and between 2 genetically divergent populations of the brook stickleback, *Culaea inconstans*. The brook stickleback is an ideal candidate for a study of this nature because recent mitochondrial DNA (mtDNA) phylogeographic analyses have identified 2 distinct allopatric lineages that began to diverge 3.6–4.8 million years (Gach 1996; Mattern 2006), thus providing an interesting historical context in which to investigate the evolution of mating signals. Male courtship is complex in the brook stickleback, consisting of at least 7 sexual and aggressive displays (Winn 1960; Reisman and Cade 1967; McKenzie 1969a, 1969b). In addition, in most populations, courting males express ephemeral, highly intense, melanic body coloration (Winn 1960; Reisman and Cade 1967; McLennan 1993). A moderate amount of behavioral variability in male sexual and aggressive signaling has been documented between individual populations (Burks et al. 1985; Ward and McLennan 2006, 2008; McLennan and Ward 2008); however, virtually nothing is known about female preferences for male courtship signals in this species. This paucity of information is surprising because female choice is highly active in brook sticklebacks; receptive females willingly deposit their eggs in the nest of a chosen male (Ward and McLennan 2008) and males perform the entirety of parental care (McKenzie 1974). Because females are presumably under strong selection to accurately predict male parental ability, female choice is a potentially important evolutionary force influencing divergence and reproductive isolation between populations.

Although visual mate assessment has never been empirically examined in the brook stickleback, female mate choice in the closely related three-spine stickleback, *Gasterosteus aculeatus*, has been well studied. Within populations, female threespines that are offered males differing in only one trait generally prefer the more intensely colored (reviewed in McLennan 2006) or larger suitor (Moodie 1982; Rowland 1989; Kraak

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et al. 1999). The relationship between male courtship effort and female choice is more complicated, with females from some populations demonstrating little preference for increased behavioral vigor and others showing strong preferences (Ward and FitzGerald 1987; Milinski and Bakker 1990; Rowland 1995; Candolin 1997; Cubillos and Guderley 2000; Künzler and Bakker 2001; Boughman et al. 2005). Cumulatively, these studies suggest that a preference hierarchy exists in the three-spine stickleback, with color and size having a stronger effect on female choice decisions than courtship behavior (e.g., Künzler and Bakker 2001; Boughman et al. 2005). Given this information, our aims in this study are 2-fold: 1) to delineate female preferences for multiple male visual cues in allopatric populations of *C. inconstans* from 2 genetic lineages and 2) to measure female responses to native versus foreign males using reciprocal mate choice tests, to examine how intrapopulation female preferences for male cues influence mate choice patterns between populations.

MATERIALS AND METHODS

Adult brook sticklebacks were collected in minnow traps from Sutherland Creek, Nebraska (41°08'13"N, 101°07'28"W), and Tooley Creek, Ontario (43°52'N, 78°46'W), in May 2005–2007. The fish were immediately transported to the University of Toronto where they were maintained under winter conditions (12:12 h light:dark regime, 10°C) in stock tanks equipped with the continual flow-through of dechlorinated water. Between May and July, individuals were removed from the winter holding tanks and placed in 120-l single-population community tanks. To promote breeding, these tanks were supplied with an abundance of live plants, sphagnum moss, and algae and were maintained at optimal summer breeding conditions (16:8 h light:dark regime, 16°C; Winn 1960; Reisman and Cade 1967). Once introduced to this environment, the fish usually became reproductively active within 3 days.

Experimental set-up and procedure

We conducted 4 sets of mate choice trials (within and between populations) from June to August of 2005–2007. In total, we examined the responses of 142 females to 62 males that exhibited 4 composite, multidimensional male visual traits encompassing a wide range of signal variability. Experiments were conducted in 120-l experimental aquaria lined with 2.5 cm of fine gravel and covered on the back and sides with green paper and photographs of aquatic vegetation. The aquaria were divided into 3 compartments by clear Plexiglas barriers. Each distal chamber was fitted with a 2.4 × 1.5-cm cylindrical aeration system and supplied with a *Hygrophila* plant rooted in a peat-filled 10-cm diameter plastic dish, as well as an abundance of soft algae and grass nesting material collected from a nearby marsh. Illumination was provided by two 11-W fluorescent bulbs suspended 10 cm above the water surface of each tank.

Males that showed evidence of reproductive motivation (i.e., nest-building behavior) were selected at random from the community tanks and measured for standard length (tip of snout to the rounded end of the caudal peduncle). Each male was then introduced into a distal compartment and permitted to build a nest. Only males that had started nest building, but had not yet received a clutch of eggs in the community tanks, were selected as stimulus males to control for any potential effects of prior mating. The opposing males were chemically and visually isolated from the central compartment and from each other via removable, white, opaque dividers fitted over the clear Plexiglas barrier. To minimize disturbance, a green curtain was hung in front of each tank. All the males used in this study

began nest building within 48 h of introduction to the test aquaria, and all built their nests in the *Hygrophila*.

Ovulated, sexually receptive females were identified in the community tanks by the expression of female nuptial coloration (McLennan 1994, 1995; Ward and McLennan 2006). To begin each trial, a female was introduced into the central compartment of a test aquarium and allowed to acclimatize to her surroundings for 10 min. After acclimation, the female was presented with the stimulus males via the removal of the opaque dividers.

For scoring purposes, the central compartment of the experimental tank was divided into three 12.5-cm zones. The 2 zones that were closest to the flanking stimulus males were designated as “preference zones” and the central zone was designated as the “neutral zone.” We recorded female response behaviors in the preference zones only (a conservative measure of choice). Trials were discarded if a test female lost nuptial coloration at any point during the observation period or if one of the males was not visible to the female (e.g., hiding in the nest plant). To control for confounding effects of familiarity, females were not presented with males with whom they had shared a community tank. The interactions between all 3 individuals were videotaped from behind a blind for 5 min. Behaviors were scored from the tapes at a later date.

Behavioral variables scored

We recorded several measures of female choice including 1) the amount of time that females spent within 12.5 cm of each stimulus male, 2) the frequency and duration of interaction bouts with the glass divider, and 3) the frequency and duration of head-up courtship displays (the female hovers stationary in a 45° angle relative to the substrate) and ground-sink bouts (the female sinks from a head-up display to the substrate and remains motionless). Head-up is the predominant courtship display shown by female brook sticklebacks, and few ground-sink bouts were observed during experimental trials (intrapopulation: 1 out of 30 females [Tooley Creek], 11 out of 35 females [Sutherland Creek]; interpopulation trial: 12 out of 77 females [both populations]). Where applicable, a single overall measure of courtship behavior was calculated for each female based on the additive frequencies of both displays.

We recorded the intensity of 16 visual signal components expressed by stimulus males in each trial. These signal components corresponded to 3 composite signal categories: nuptial coloration, sexual courtship, and aggressive courtship. Details of color scoring can be found in McLennan (1993) and Ward and McLennan (2006). The intensities of body color, dorsal, caudal and anal fin color, dorsal spine color, and the development of vertical eye bars were scored immediately after each presentation. For body color, an intensity value ranging from 0 (least intense) to 10 (most intense) was assigned more than 5 bodily regions by comparing body hue with the grayscale color series in the *Naturalist Color Guide* (Smithe 1975). Summing the values produced an overall color value ranging from 0 (no color) to 50 (intensely black). Fin color and spine color were recorded on a scale of 0–2 corresponding to no color (0), speckled melanophore distribution (1), and solid black color (2). Finally, eye bar development was assigned a value from 0 (no eye bar) to 6 (entirely black).

The frequencies of 3 sexual displays (pummel, waggle dance, and nest lead) and 3 aggressive displays (bite, broadside threat, tailbeats) were scored following Ward and McLennan (2006, 2008) and McLennan and Ward (2008) (Table 1). Biting is the only aggressive behavior that typically terminates in harmful contact between free-swimming fish; the other 2 displays are ritualized threat displays that do not result in harm to the female and are referred to herein as “ritualized courtship

Table 1
Descriptions of aggressive and sexual displays performed by stimulus male brook stickleback during courtship

Display	Description
Sexual	
Pummeling	From a position slightly above, and approximately 10–15 cm away from, the female (McKenzie 1969a), the male dashes downward, repeatedly striking the female on her head and flanks with his snout (Hall 1956).
Waggle dance	Males assume a distinctive mode of swimming that is characterized by exaggerated lateral tail movement and an oscillating torso (Hall 1956). Although usually observed during a nest-leading episode, males may occasionally waggle dance during pre-lead courtship interaction.
Nest lead	Male swims toward the nest and then pauses above or beside it. The swim may be direct or circuitous and may or may not be interrupted by pauses. The female may or may not follow the male during the nest lead.
Aggressive	
Broadside threat	The male assumes a horizontal, concave posture, displaying laterally to the opposing fish. The dorsal and pelvic spines are often flared and fins erected.
Tailbeating	The male beats his tail laterally in a slow (approximately 4–10 beats per second), exaggerated manner (McKenzie 1969b).
Bite	The male attempts to bite the female through the glass partition.

aggression.” Biting attacks were very infrequent during trials and were not correlated with ritualized threat displays (Table 2), suggesting that threat displays do not indicate intersexual agonistic motivation in the context of courtship. Separate preliminary regression analyses of biting attempts on female response were not significant within either population (Tooley Creek females: adjacent [adj.] $r^2 = -0.03$, $P = 0.61$; Sutherland Creek females: adj. $r^2 = 0.02$, $P = 0.20$). Therefore, for statistical

purposes, we used the correlated suite of traits broadside and tailbeat as our measure of ritualized courtship aggression.

Mate choice trials

Intrapopulation choice trials

In all, 30 Tooley Creek females and 35 Sutherland Creek females were tested for visual trait preferences within their respective populations using 7 Tooley Creek and 8 Sutherland Creek male stimulus pairs, respectively. A maximum of 5 females were tested with any given stimulus pair, and each female was only tested once. In addition to examining female preferences for male visual traits within populations, the relationships among male courtship traits were compared between populations using Spearman correlations ($n = 14$ and $n = 16$ for Tooley Creek and Sutherland Creek, respectively). Correlations were conducted on mean trait values calculated across all the trials in which each male participated (1–5 trials).

Interpopulation choice trials

In all, 39 Tooley Creek females and 38 Sutherland Creek females were tested under dichotomous (native vs. foreign stimuli) choice conditions using a total of 16 pairs of stimulus males randomized with respect to chamber position (15 and 13 pairs used for Tooley Creek and Sutherland Creek females, respectively). The relative proportions of Tooley Creek and Sutherland Creek focal females tested with each pair depended on the availability of gravid females. Females were tested once, but stimulus pairs were used with up to 8 females from a given population (maximum 9 females in total). We tested for evidence of assortative mate choice via *t*-tests conducted on female responses to paired native and foreign stimuli. We tested for population differences in female behavior toward either male using multivariate analyses of variance (MANOVAs) with female population origin specified as the fixed factor and preference measures (association time, interaction duration, intensity of courtship response) specified as dependent variables. We calculated the mean population preference strength for native stimuli according to the equation:

Table 2
Spearman correlations of phenotypic courtship traits observed in male brook sticklebacks from Tooley Creek, Ontario ($n = 14$), and Sutherland Creek, Nebraska ($n = 16$)

	Nuptial Color					Courtship			Aggression			Size	
	Body ^a	Dorsal fin	Anal fin	Caudal fin	Dorsal spine	Eye bar	Pummel	Waggle dance	Nest lead	Broadside threat	Tailbeat	Bite	Body size
Body ^a	—	0.84***	0.79***	0.70**	0.81***	0.85***	0.78***	0.76**	0.73**	-0.07	0.01	0.23	0.09
Dorsal fin	0.75**	—	0.98***	0.83***	0.75**	0.76**	0.87***	0.86***	0.91***	-0.24	-0.19	-0.00	0.08
Anal fin	0.84**	0.81***	—	0.85***	0.74**	0.74**	0.87***	0.82***	0.89***	-0.24	-0.19	-0.06	0.09
Caudal fin	0.53	0.72**	0.67**	—	0.67**	0.63**	0.68**	0.58*	0.62*	-0.25	-0.32	0.12	0.08
Dorsal spine	0.88***	0.92***	0.86***	0.69**	—	0.73**	0.66**	0.66**	0.57*	0.08	0.13	-0.04	-0.07
Eye bar	0.88***	0.71**	0.81***	0.66*	0.80*	—	0.82***	0.74**	0.72**	0.15	0.10	0.23	-0.01
Pummel	0.67**	0.45	0.65*	0.60*	0.57*	0.88***	—	0.84***	0.92***	-0.12	-0.18	0.13	0.11
Waggle	0.38	0.36	0.48	0.62*	0.42	0.57**	0.90***	—	0.94***	-0.13	-0.14	-0.05	0.25
Nest lead	0.43	0.35	0.48	0.61*	0.44	0.72**	0.93***	0.97***	—	-0.18	-0.16	-0.03	0.24
Broadside	0.41	0.13	0.42	-0.04	0.31	0.50	0.49	0.28	0.31	—	0.83***	0.46	-0.26
Tailbeat	0.44	0.28	0.57*	0.10	0.40	0.50	0.45	0.25	0.27	0.91***	—	0.27	-0.40
Bite	0.25	0.41	0.45	0.60*	0.38	0.52	0.44	0.45	0.34	0.33	0.47	—	-0.31
Body size	0.22	0.14	0.31	0.43	0.03	0.30	0.34	0.33	0.23	-0.13	-0.03	0.57*	—

Correlations were computed using average values across trials for each male. Sutherland Creek, upper right of diagonal, Tooley Creek, lower left of diagonal.

^a Additive score based on 5 body regions.

*Significance at alpha < 0.05; **significance at alpha < 0.01; ***significance at alpha < 0.001.

$$q_2 = (t_N - t_F)/(t_N + t_F),$$

where t = time and subscripts N and F are native and foreign males, respectively. According to this equation, -1 represents complete preference for the foreign stimuli and 1 represents complete preference for the native stimuli. A difference in the mean strength of preference for native mates was tested via analysis of variance (ANOVA).

Female preferences for multiple male traits

Multiple regressions of female preference strength (q_1) on visual trait differentials between stimulus males ($[z]$ see below) were used to determine the relationship between female mate choice and male courtship traits within and between populations. Because female preference measures were highly correlated in both populations, we generated preference indices based on measures of time association. We calculated the strength of preference (q_1) as the proportion of total time spent associating with the preferred male; these indices could vary between 0 (no preference for either stimulus) and 1 (complete preference for one stimulus over the other).

Corresponding male trait differentials (z = preferred male – nonpreferred male) were calculated for each trial with respect to courtship intensity, aggressive intensity, nuptial color intensity, and body size. Because the intensities of the male color components were correlated in both populations (Table 2), we initially combined the intensities of body, fin, and spine coloration (with the exception of the caudal fin) and the degree of eye bar development in a principle component analysis that represented an overall intensity score for each stimulus male in each trial. Similarly, we used principle components to derive an overall courtship score for each male based on the intensity of sexual displays (frequency/s), as well as an overall aggressive score based on the intensity of broadside threat and tailbeating displays (frequency/s). For all models, color, courtship, and aggressive trait decompositions yielded a single principle component that explained a high percentage of behavioral variation (color > 79%, courtship > 86%, aggression > 86%), and all factors were highly positively loaded on their respective components.

For comparative purposes, traits were expressed as deviations from the mean phenotype, with a mean of 0 and standard deviation of 1 (Lande and Arnold 1983); regression coefficients (β) therefore represent standardized measures of trait effects. Initial screening showed that all the regression models satisfied assumptions of linearity, and residuals were normally distributed (Kolmogorov–Smirnov test: $P > 0.05$ for all models). In all 4 models, there was no evidence for multicollinearity between independent factors (all variance inflation factors < 1.75). Separate regression equations including quadratic and cross-product terms did not reveal significant, nonlinear female responses and were not investigated further.

Although males were used more than once, stimulus trait differentials fluctuated extensively from trial-to-trial, and each test female experienced a unique set of multivariate visual differences; examination of Durbin–Watson statistics confirmed that residual errors were not autocorrelated, and thus, stimulus conditions in each trial were considered statistically independent. Nonetheless, the robustness of the regression results from the main interpopulation analysis was examined by comparing the patterns of female response with those obtained using male and female trait and response means from each male set. Although the expected large reduction in statistical power affected significance with respect to some traits in the latter analysis, no major inconsistencies between the 2 sets of data were observed. Female responses toward individual male visual traits demonstrated similar patterns of direction and either maintained significance or demonstrated near significance.

Finally, in preliminary models, we investigated the influence of heterogeneity between naturally varying stimulus pairs on female preferences by including male stimulus pair as an independent variable (Kozak et al. 2008). However, because the effect of stimulus set was not significant (all $P > 0.05$), it was removed prior to final analysis.

RESULTS

For the 4 choice series (Sutherland Creek intrapopulation, Tooley Creek intrapopulation, Sutherland Creek interpopulation, Tooley Creek interpopulation), absolute signal variation expressed by the stimulus males varied widely for each of the composite traits recorded, and females encountered a wide range of relative trait differentials between opposing stimulus males (Supplementary Material). Preliminary analysis indicated that the Sutherland and Tooley Creek males used in intrapopulation choice tests were comparable to males used in interpopulation trials; males did not differ with respect to trait means across trial series (MANOVA: Tooley Creek: $F_{5,23} = 1.88$, $P = 0.14$; Sutherland Creek: $F_{5,23} = 0.30$, $P = 0.91$). Furthermore, with the exception that Tooley Creek females encountered a larger range of aggressive trait differentials in the intrapopulation choice context (Levene's: $F_{1,67} = 17.91$, $P < 0.001$), variation in stimulus male trait differentials around the respective means was, for both populations of females, approximately equal between choice contexts.

Intrapopulation choice trials

Relationships among composite male visual traits

Spearman rank correlations between male courtship traits revealed similar patterns of trait associations in males from the 2 populations. With the exception of caudal fin color, the intensities of male color components (body, eye, fins, spines) were positively correlated in both populations (Tooley Creek: $r = 0.71$ – 0.92 ; Sutherland Creek: $r = 0.73$ – 0.98), as were the 3 sexual behaviors (Tooley Creek: $r = 0.90$ – 0.97 ; Sutherland Creek: $r = 0.84$ – 0.94), and the intensities of broadside and tailbeating aggressive threat displays (Tooley Creek: $r = 0.91$; Sutherland Creek: $r = 0.83$). Overall, courtship behaviors were moderately correlated with color components in both populations, and neither the intensity of nuptial color components nor the intensity of courtship displays was correlated with the intensity of aggressive displays (Table 2).

*Female preferences for male visual traits in 2 populations of *C. inconstans**

Based on the assessment of visual traits, females within both the populations tested made clear choices between available stimulus males (Tooley Creek female: adj. $r^2 = 0.34$, $F_{4,25} = 4.77$, $P = 0.005$; Sutherland Creek female: adj. $r^2 = 0.43$, $F_{4,30} = 7.44$, $P < 0.001$; Table 3). The primary criterion of mate assessment was courtship behavior (Tooley Creek: $\beta = 0.50$, $P = 0.02$; Sutherland Creek: $\beta = 0.53$, $P = 0.001$; Table 5), and plotting female response as a function of relative courtship effort revealed that q_1 was positively related to z in both populations (Figure 1). In contrast, nuptial color intensity, body size, and the intensity of ritualized courtship aggression were not significant predictors of female mate choice in either population (Table 5).

To examine whether females from Tooley Creek and Sutherland Creek differ in their response to individual male traits, an analysis of covariance (ANCOVA) was performed including female population source as a fixed factor and trait differentials as covariates. All main effects and trait \times population interactions were included in the model. Variation between the populations in the strength of female

Table 3

Behavioral responses of female brook stickleback (mean \pm standard error) from Tooley Creek, Ontario ($n = 30$), and Sutherland Creek, Nebraska ($n = 35$), toward preferred and nonpreferred stimuli males during intrapopulation dichotomous choice mating trials

Female	Tooley Creek				Sutherland Creek			
	Preferred	Nonpreferred	Paired <i>t</i> -test		Preferred	Nonpreferred	Paired <i>t</i> -test	
Male			t_{29}	<i>P</i>			t_{34}	<i>P</i>
Total duration (s)								
Time spent near	209.12 (11.41)	53.85 (7.46)	8.51	<0.001	192.17 (7.52)	73.11 (6.15)	9.09	<0.001
Total interaction	72.48 (10.85)	12.92 (2.72)	5.22	<0.001	65.72 (7.37)	19.33 (2.54)	5.65	<0.001
Total courtship display	18.59 (5.28)	9.36 (8.20)	0.90	0.380	16.96 (4.63)	4.71 (1.69)	2.71	0.010
Bout duration (s)								
Interaction bout	7.10 (0.79)	4.91 (1.10)	2.07	0.048	7.11 (0.68)	4.35 (0.59)	2.84	0.008
Courtship display bout	9.19 (2.51)	0.62 (0.40)	3.38	0.002	7.20 (1.86)	1.87 (0.62)	2.84	0.008
Frequency								
Courtship display	1.77 (0.41)	0.17 (0.10)	3.86	0.001	1.63 (0.36)	0.71 (0.22)	2.78	0.009
Approach	10.00 (1.35)	2.13 (0.38)	5.14	<0.001	9.20 (0.80)	4.00 (0.50)	5.61	<0.001

“Winning” and “losing” males were determined on the basis of side association data.

preference for the chosen male was detected ($F_1 = 4.41$, $P = 0.04$); however, the effect size was negligible (Wilks’ partial variance statistic [η^2] = 7%). In addition, we found no evidence of slope heterogeneity between populations for any composite male trait. In other words, Tooley Creek and Sutherland Creek females respond similarly to each of the male signals recorded (Table 6).

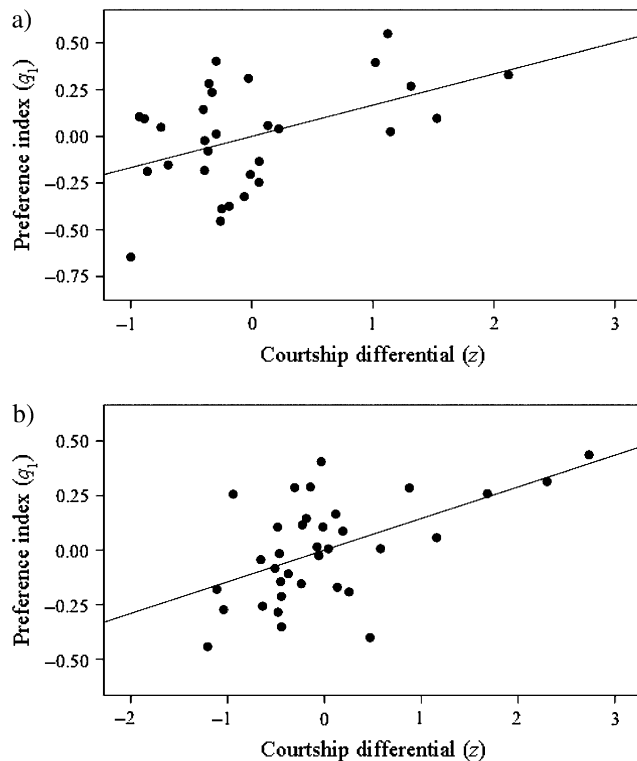


Figure 1
Partial regression plots of the relationship between standardized relative courtship differentials (z) and female preference strength (q_1) for females from 2 populations of brook stickleback from (a) Tooley Creek, Ontario, and (b) Sutherland Creek, Nebraska. Both axes are expressed as residual values after accounting for variation in female responses due to color, aggressive intensity, and size.

Interpopulation choice trials

Assortative mate choice between populations

Consistent with previous work (Ward and McLennan 2006), the 16 Sutherland Creek males used in this experiment were, on average, ~15% lighter in their expression of nuptial color intensity than the 16 experimental Tooley Creek males (mean score: 22.95 vs. 30.50; paired *t*-test: $t_{15} = -1.89$, one-tailed $P = 0.04$) and ~13% larger (mean: 46.82 vs. 40.74 mm; paired *t*-test: $t_{15} = 4.97$, $P < 0.001$). Sutherland Creek males also courted with an overall intensity that was 3 times that of Tooley Creek males (mean total courtship: pummel + waggle + lead: 14.67 vs. 5.53 per trial; paired *t*-test: $t_{15} = 2.41$, $P = 0.03$). The average levels of ritualized aggressive threat displays were similar between populations (broadside threat + tailbeating bouts: Sutherland Creek: 3.39 vs. Tooley Creek: 4.60 per trial; paired *t*-test: $t_{15} = -0.63$, $P = 0.54$).

When presented with native and foreign males, females from Sutherland Creek and Tooley Creek demonstrated differential patterns of response (q_2 : ANOVA: $F_1 = 13.67$, $P < 0.001$; Figure 2). In total, 26 of 38 (68%) Sutherland Creek females spent more time associating with native than foreign males; however, only 11 of 39 Tooley Creek females (28%) showed a preference for the native male (Table 4). Females from both populations spent more time in the preference zone closest to the Sutherland Creek male (Tooley Creek: $t_{38} = 2.74$, $P = 0.009$; Sutherland Creek: $t_{37} = 2.43$, $P = 0.02$) and more time interacting with the Sutherland Creek male (Tooley Creek: $t_{38} = 2.40$, $P = 0.02$; Sutherland Creek: $t_{37} = 2.64$, $P = 0.01$). Females also approached the Sutherland Creek male more often (Tooley Creek: $t_{38} = 2.40$, $P = 0.02$; Sutherland Creek: $t_{37} = 3.45$, $P = 0.001$). Tooley Creek females spent more time engaged in courtship displays ($t_{38} = 2.11$, $P = 0.04$) and performed longer mean interaction bouts with the foreign (Sutherland) male ($t_{38} = 2.40$, $P = 0.02$). Females from the 2 populations responded with similar intensity toward both the Sutherland Creek and Tooley Creek males (MANOVA: Wilks’ lambda = 0.82, $F_{12,64} = 1.20$, $P = 0.31$).

Interpopulation mate assessment based on visual traits

According to multivariate regression, interpopulation mate choice was related to visual trait differences between the stimulus males in the traits measured (Tooley Creek female: adj. $r^2 = 0.34$, $F_{4,34} = 5.91$, $P = 0.01$; Sutherland Creek female: adj. $r^2 = 0.24$, $F_{4,33} = 3.97$, $P = 0.01$) and indicated that female interpopulation mate choice in the brook stickleback is

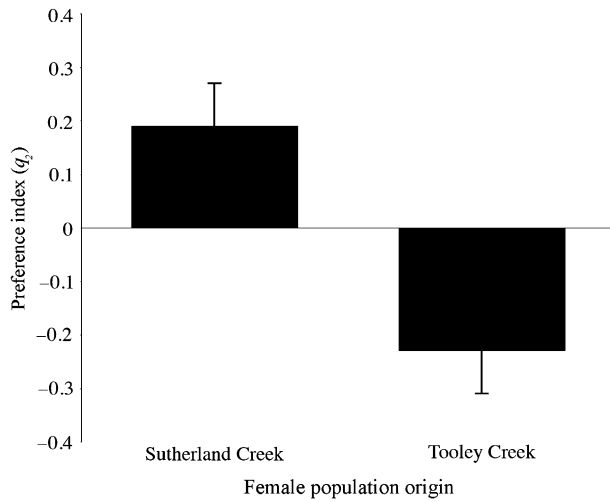


Figure 2 Asymmetric mate choice in 2 populations of brook stickleback from Sutherland Creek, Nebraska, and Tooley Creek, Ontario. Bars represent the mean strength of preference for local males ($q_1 \pm$ standard error).

largely governed by the same assessment criteria used to make intrapopulation mating decisions. Consistent with results of the intrapopulation mate choice trials, all females favored of the higher courting male (Tooley Creek: $\beta = 0.39$, $P = 0.009$; Sutherland Creek: $\beta = 0.62$, $P = 0.003$; Table 5, Figure 3). Neither population of females discriminated between available mates on the basis of the relative intensities of nuptial color or size (Table 5). Tooley Creek females discriminated between males on the basis of aggressive intensity ($\beta = 0.50$, $P = 0.002$; Table 5), but overlapping confidence intervals suggested that the slope of the female response to ritualized courtship aggression was not significantly different between the populations.

To examine whether females altered mate assessment behavior between choice contexts, ANCOVAs were run for each population specifying trial type as a fixed factor (intra- vs. interpopulation) and trait differentials as covariates; all main effects and trait \times trial type interactions were included in the model. With the single exception of a significant size \times trial type interaction in the Tooley Creek population ($F_1 = 4.51$, $P = 0.04$), we found no evidence of slope heterogeneity

between choice contexts in either population. Thus, regardless of whether males were local or foreign, females discriminated between available mates in a similar manner on the basis of visual cues (Table 6).

DISCUSSION

Our results indicate that courtship effort, as determined by overall display rate, is the primary visual criterion of mate assessment in female brook stickleback; females from 2 geographically disjunct populations exhibited directional preferences for increased behavioral vigor and maintained similar female response functions across different mate choice contexts (intra- vs. interpopulation). In contrast, the magnitude of the difference between stimulus males in nuptial color intensity and body size had little influence on mate choice decisions. Female mate assessment was highly conservative between the 2 genetically and geographically disparate populations that we tested. Conservation of mate assessment strategies, coupled with divergence in male courtship traits, generated asymmetric patterns of assortative mate choice between the populations.

Female preferences for male traits in *C. inconstans* and other sticklebacks

Male parental care in sticklebacks is energetically costly (reviewed in Wootton 1994; Östlund-Nilsson 2006). Egg aeration and nest defense significantly reduce male energetic reserves (FitzGerald et al. 1989; Chellappa and Huntingford 1989; Smith and Wootton 1999), which can decline as much as 50% over the course of the breeding season (Chellappa et al. 1989). Because egg production by females is also energetically expensive (reviewed in Wootton et al. 1995), females are presumably under strong selection to accurately anticipate a given male's ability to meet the demands of fatherhood. Rapid display movements, such as those that characterize the courtship activities of male sticklebacks, are generally costly to perform and may reliably advertise a courting male's condition (Rowland 1995). Indeed, in many teleost fishes with paternal care, courtship intensity (sexual and/or aggressive) positively correlates with parental effort and offspring survival (e.g., damselfish: Knapp and Kovach 1991; sunbleak: Gozlan et al. 2003). For example, in the fifteen-spined stickleback, *Spinachia spinachia*, females prefer males that demonstrate higher rates of the body shake courtship display, and males with higher shaking rates experience greater hatching success

Table 4

Behavioral responses of female brook stickleback (mean \pm standard error) from Tooley Creek, Ontario ($n = 39$), and Sutherland Creek, Nebraska ($n = 38$), toward males from their own population (local mate) and an unfamiliar population (foreign mate), during dichotomous choice trials

Female	Tooley Creek (Ontario)				Sutherland Creek (Nebraska)			
			Paired <i>t</i> -test				Paired <i>t</i> -test	
Male	Tooley Creek	Sutherland	<i>t</i> ₃₈	<i>P</i>	Tooley Creek	Sutherland	<i>t</i> ₃₇	<i>P</i>
Total duration (s)								
Time spent near	98.63 (10.98)	160.79 (12.06)	2.74	0.009	108.26 (10.60)	160.58 (11.20)	2.43	0.020
Total interaction	27.28 (4.89)	49.33 (6.12)	2.40	0.020	29.69 (3.76)	46.92 (5.46)	2.64	0.010
Total courtship display	14.20 (3.69)	30.46 (7.83)	2.11	0.040	13.39 (6.72)	16.00 (4.34)	0.34	0.740
Bout duration (s)								
Interaction bout	4.49 (0.50)	5.93 (0.47)	2.40	0.020	5.10 (0.55)	5.44 (0.44)	0.59	0.560
Courtship display bout	5.13 (1.35)	13.53 (4.70)	1.69	0.100	5.32 (2.04)	6.10 (1.35)	0.34	0.740
Frequency								
Courtship display	1.08 (0.27)	1.51 (0.25)	1.35	0.180	0.71 (0.20)	1.32 (0.33)	1.79	0.080
Approach	4.90 (0.83)	8.31 (0.93)	2.40	0.020	4.97 (0.55)	8.05 (0.76)	3.45	0.001

Table 5**Multiple linear regression assessing the independent effects of multiple male visual traits on female mate choice in 2 populations of brook stickleback**

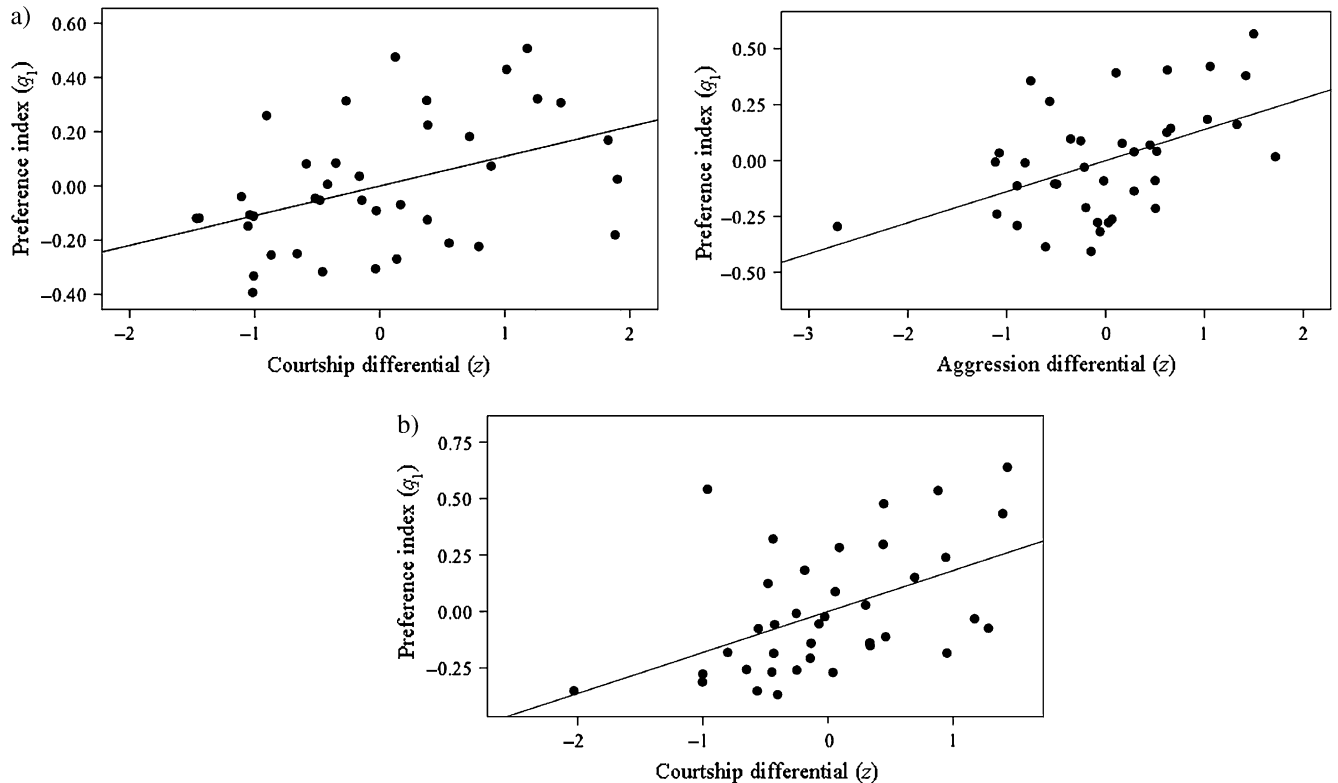
Trait	Tooley Creek			Sutherland Creek		
	β	t	P	β	t	P
Intrapopulation mate assessment						
Color	0.12 (-0.28-0.52)	0.60	0.55	0.26 (-0.07-0.60)	1.61	0.120
Courtship	0.50 (0.11-0.90)	2.61	0.02	0.53 (0.22-0.83)	3.55	0.001
Aggression	0.23 (-0.12-0.64)	1.40	0.18	0.09 (-0.21-0.39)	0.58	0.560
Size	-0.28 (-0.66-0.10)	-1.51	0.14	0.05 (-0.21-0.32)	0.42	0.680
Interpopulation mate assessment						
Color	-0.07 (-0.36-0.22)	-0.49	0.630	-0.05 (-0.37-0.27)	-0.35	0.731
Courtship	0.39 (0.11-0.68)	2.79	0.009	0.61 (0.23-0.99)	3.24	0.003
Aggression	0.50 (0.20-0.80)	3.28	0.002	0.17 (-0.14-0.49)	1.10	0.278
Size	0.21 (-0.09-0.52)	1.44	0.160	-0.22 (-0.60-0.16)	-1.18	0.248

Standardized β values reflect the relationship between visual trait differences between stimulus males and the strength of female preference (q_1) for the winning male. Values in parentheses indicate 95% confidence intervals.

(Östlund and Ahnesjö 1998; Östlund-Nilsson and Mayer 2006). Our finding that courtship intensity is the primary determinant of mate choice in the brook stickleback is consistent with these reports, and future research may uncover a similar relationship between courtship intensity, parental care, and offspring survival in this species.

In contrast to strong responses to courtship vigor, the female *C. inconstans* that we tested did not demonstrate a mating bias toward larger males. This result was initially unexpected given the difference between the populations in mean male size (46.82 vs. 40.74 mm), and the general observation that females in many fish species with paternal care tend to prefer bigger

males. However, our results are similar to those reported for the fifteen-spined stickleback. In this species, females do not discriminate among males based on body size, and furthermore, no correlation exists between male size and hatching success (Östlund and Ahnesjö 1998). In both *C. inconstans* and *S. spinachia*, female mate assessment is consistent with the general prediction that, when a male's energetic state is subject to environmental and social variation (e.g. food availability, disease, dominance hierarchy), females concerned with estimating male condition at the time of mating should prioritize the assessment of transient signals that covary with condition (e.g., display rate, color brightness) over fixed male

**Figure 3**

Partial regression plots for significant relationships between interpopulation trait differentials (z) and female preference strength (q_1) for females from 2 populations of brook stickleback from (a) Tooley Creek, Ontario, and (b) Sutherland Creek, Nebraska. Both axes are expressed as residual values after accounting for variation in female responses due to the other male traits measured.

Table 6

Results of ANCOVA examining differences in female mate assessment between populations within intrapopulation choice contexts (population: $F_{9,55} = 5.74$, $P < 0.001$) and for individual populations under intra- versus interpopulation choice contexts (Tooley Creek: $F_{9,59} = 4.97$, $P < 0.001$; Sutherland Creek: $F_{9,63} = 4.75$, $P < 0.001$)

Factor	Population		Intra vs. interpopulation choice			
			Tooley Creek		Sutherland Creek	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Trial type/population	4.41	0.040	1.60	0.210	0.43	0.520
Color	2.09	0.150	0.07	0.790	0.72	0.430
Courtship	18.11	<0.001	14.67	<0.001	22.37	<0.001
Aggression	2.36	0.130	9.61	0.003	1.43	0.240
Size	1.33	0.250	0.22	0.639	0.59	0.450
Trial type/population × aggression	0.77	0.390	0.53	0.470	0.19	0.660
Trial type/population × color	0.19	0.670	0.64	0.429	1.78	0.190
Trial type/population × courtship	0.09	0.770	0.64	0.430	0.28	0.600
Trial type/population × size	2.53	0.120	4.51	0.040	1.52	0.220

characteristics (e.g., size, color pattern) (Haley et al. 2004; see also Barbosa and Magurran 2006). Even when Tooley Creek females were presented with males larger than those encountered within the normal range of size variation (interpopulation choice context), male preference was not related to differences in size; because larger males did not court more vigorously than smaller males in either population (Table 2), and females from the 2 populations responded similarly to courtship vigor, it is likely that interpopulation differences in male size have been shaped by either differential natural selection pressures (e.g., due to differential predation regimes; Langerhans et al. 2007) or variation in the strength of intrasexual selection acting on male dominance traits (Rowland 1989; Dufresne et al. 1990; Kraak et al. 2000; Östlund-Nilsson 2000) rather than by direct intersexual selection or correlational selection on female preferences for increased rates of courtship.

The patterns of predominant mate assessment criteria in *C. inconstans* and *S. spinachia* differ from those of the three-spined stickleback, *G. aculeatus*, a species in which the relationship between courtship effort and female choice is variable across populations (Ward and FitzGerald 1987; Milinski and Bakker 1990; Rowland 1995; Candolin 1997; Cubillos and Guderley 2000; Künzler and Bakker 2001; Boughman et al. 2005), and mate choice within populations is often strongly based on nuptial color intensity and body size (reviewed in McLennan 2006, Östlund-Nilsson 2006). Indeed, between populations or ecotypes, assortative mating based on ecotype-specific size matching is frequently found to be a driving force maintaining reproductive isolation (McKinnon et al. 2004; Boughman et al. 2005), with differences in color expression also playing a role (Boughman et al. 2005). Notably, our results indicate that the intensity of melanin, sexually dimorphic male body color is the least important component of female choice in the brook stickleback; females did not discriminate between males on the basis of nuptial color intensity within either population nor did differences in the intensity of male nuptial coloration between populations (Ward and McLennan 2006) influence interpopulation mating dynamics.

Interspecific divergence in the importance that females place on male nuptial color as a criterion of mate choice potentially reflects the difference in the dominant pigment systems utilized by the 2 species. Because a carotenoid-based signal can divert pigments from such critical functions as the detoxification of free radicals, modulation of the immune system, and sperm protection (Blount et al. 2001; Lozano 2001), red coloration is a strong indicator of quality; only high-quality individuals should be able to pay this price (reviewed in Alonso-Alvarez et al. 2004). Accordingly, in three-spine sticklebacks, red coloration has been positively associated with current body condition (e.g., Milinski and Bakker 1990; Frischknecht 1993; Bakker and Mundwiler 1994; Barber et al. 2000; Candolin 2000a, 2000b; Boughman 2007), behavior performed during parental care (McKinnon 1996; Candolin 2000a) and, ultimately, female preference (McLennan 2006; Östlund-Nilsson 2006).

Although a positive relationship between carotenoid-based body color and condition appears to be general in nature (Griffith et al. 2006), comparative evidence suggests that the relationship between melanin male ornaments and condition might be taxonomically and ecologically specific (Stoehr 2006). In vertebrates, melanins are internally synthesized (Hearing 1993) and are extensively integrated with other organismal functions (Badyaev and Young 2004 and references therein). Both of these qualities limit their sensitivity to environmental conditions (Gray 1996; Badyaev et al. 2002; Badyaev and Hill 2003). Overall, there is limited empirical evidence to suggest that melanin-based signals reflect condition or quality to the same extent as carotenoid-based signals (e.g., Badyaev and Hill 2000; McGraw et al. 2002; Senar et al. 2003; Stoehr 2006; but see Griffith et al. 2006). Therefore, it is not surprising that few studies have documented a role for melanin in female choice (Møller 1990; Norris 1990; Veiga 1993; Badyaev and Hill 2000). Under laboratory conditions, most brook stickleback males are capable of achieving maximal black coloration (McLennan 1993; Ward and McLennan 2006). Combined with the present study, these results imply that the melanin signal in the brook stickleback may not be tightly correlated with male quality in the same manner as is the carotenoid-based nuptial coloration of the threespine. However, male brook sticklebacks from the Tooley Creek population do increase the intensity of their signal more in response to a courting female than to an intruding territorial male (McLennan 1993), suggesting that there is some information in the signal specific to the male–female courtship dialogue. Experiments designed to further clarify the role of nuptial coloration in female choice decisions of the brook stickleback are currently being conducted.

Patterns of assortative mate choice between populations

Our results revealed a unidirectional preference for Sutherland Creek males by females of both populations, providing some evidence that any premating isolation based on visual cues between the 2 genetic lineages previously identified by restriction fragment length polymorphism, and mtDNA phylogeographic analysis is likely to be incomplete (Gach 1996; Mattern 2006). Theory predicts that females will prefer native males over foreign males whenever male signals and female preferences for those signals have diverged in a predictable, coordinated manner (Lande 1981). However, a lack of strong covariation in trait–preference divergence can lead to asymmetrical female mating decisions or a preference for a foreign mate (Schwartz and Hendry 2006). One mechanism by which such a phenomenon can arise is if females exhibit a generalized, linear preference for male signals outside the normal range (reviewed in Ryan and Keddy-Hector 1992; Price

1998; Sherman and Reeve 1999). Because females from the 2 genetically divergent populations of brook stickleback that we tested showed open-ended directional preferences for increased rates of courtship, it is likely that this dynamic was responsible for the observed patterns of asymmetric assortative female mate choice that we uncovered. The shapes of the brook stickleback female preference functions for courtship were similar, not only between intrapopulation and interpopulation dichotomous testing regimes but also between populations within a given mate choice context. Thus, although natural selection appears to have directly or indirectly (e.g., through trait correlation) constrained the rate of male courtship behavior to differing extents (Sutherland Creek males court approximately 3 times more intensely than Tooley Creek males), sexual selection has maintained a universal female preference for males with higher rates of courtship display, regardless of group affiliation and any stochastic effects of geographic isolation (Arnold et al. 1996).

A mechanistic understanding of how sexual selection operating within populations influences mating dynamics and reproductive isolation at higher levels of organization has received a great deal of recent interest (West-Eberhard 1983; Ptacek 2000; Panhuis et al. 2001; Kirkpatrick and Ravigne 2002; Ritchie 2007 and references therein), and directional intraspecific female preferences for specific traits have been implicated as causal factors leading to heterospecific mate preferences in a number of taxa (e.g., Morris et al. 1978; Ryan and Wagner 1987; Kozak et al. 2008). The present findings are consistent with the hypothesis that within-population trait preferences direct intergroup mating dynamics (Ryan 1990; Wiernasz and Kingsolver 1992; Langerhans et al. 2007) and highlights the importance of diagnosing the shape and form of within-population female preferences for complex signals, in addition to quantifying the extent of divergence in male traits, in studies designed to understand the causal mechanisms behind mating dynamics at higher levels of organization.

SUPPLEMENTARY MATERIAL

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

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