

Male competition and female choice interact to determine mating success in the bluefin killifish

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Whether male competition and female choice act in concert, independently, or in opposition is a critical issue for understanding sexual selection. In complex social systems, the outcomes of pairwise interactions may not be accurate indicators of how sexual selection emerges. We investigated how female choice and male competition interact in the bluefin killifish, *Lucania goodei*, in a 3-staged experiment where 1) females could choose between 2 males, 2) those males could interact in the presence of that female, and 3) females and males could freely interact and spawn. In the pairwise stages (1 and 2), females displayed pronounced preferences between males and male competition produced a distinctly dominant individual. None of the morphological traits, including color, measured in males were associated with either female preference or male dominance. When all 3 fish interacted (stage 3), male activity level was the sole predictor of spawning success. Males with elevated activity levels were more aggressive toward males and females, exhibited intensified courtship, and obtained more spawns. Female preference did not predict the number of spawns with a male, but it did predict her latency to spawn; females spawned more quickly with preferred males. Thus, male competition and female choice interact to determine reproductive success, but there is evidence for conflict and a cost to females of associating with dominant males. Reproductive success in this species is not easily predicted from simple measures of morphology or female preference and is influenced by complex social interactions, both between males, and between males and females. *Key words*: aggressive behavior, dominance status, *Lucania goodei*, sexual conflict, sexual selection, social interactions. [*Behav Ecol* 18:822–830 (2007)]

Sexual selection unfolds through the net results of interactions among males for access to females and the choices that females make among males to which they are exposed. The critical issue for understanding sexual selection in more complex social systems is whether male–male competition and female choice act in concert, independently, or in opposition (Berglund et al. 1996; Wiley and Poston 1996; Wong and Candolin 2005).

Understanding sexual selection is relatively easy when the 2 components act in concert and favor the elaboration of the same traits. In these cases, a sexually selected trait may serve both as an armament in male competition and as an ornament in female mate choice (Berglund et al. 1996; Wiley and Poston 1996). Female preference for winners of male competitive interactions may arise because dominance acts as a reliable signal of high quality and/or because dominant males offer potential direct and indirect benefits to females (Qvarnström and Forsgren 1998). There are a number of cases in which female choice is based on male dominance and/or traits associated with male dominance status (e.g., Candolin 1999, 2000; Mateos and Carranza 1999; Doutrelant and McGregor 2000; Berglund and Rosenqvist 2001; Kortet and Hedrick 2005). In some mating systems, females incite male competition in order to elucidate dominance status and ensure mating with dominant males (e.g., Cox and LeBoeuf 1977; Hovi et al. 1995; Pizzari 2001).

Understanding sexual selection as an evolutionary force is more complicated when male competition and female choice act independently; that is, when the outcome of male competition is based on one trait, whereas female choice is based on

another (e.g., Howard et al. 1997; Petersson et al. 1999; Pryke et al. 2001; Andersson et al. 2002; Candolin 2005). In this situation, even though the 2 components act independently, the evolutionary outcome will depend on the genetic correlations between the trait values favored by each component.

The most challenging scenario is when male competition and female choice act in opposition. In the simpler of these cases, male interactions and female choice select in opposite directions on the same trait (e.g., Moore AJ and Moore PJ 1999; Moore et al. 2001; Sih et al. 2002; Bonduriansky and Rowe 2003; Ophir and Galef 2003; Candolin 2004), and the net result will reflect the balance between those agents. In the more intricate cases, each agent of selection acts on multiple traits, and phenotypic correlations among traits create complicated patterns of direct and indirect selection (Qvarnström and Forsgren 1998; Wong and Candolin 2005). For example, large males may dominate smaller males, but females may choose mates based on the paternal care provided to their offspring and thus indirectly select for smaller, subordinate males that are better fathers (Forsgren 1997; Wong 2004b). Male interactions may also interfere with or overwhelm female choice making it difficult for females to assess male traits accurately or exercise choice and thereby reducing the magnitude, but not the direction, of sexual selection (e.g., Trail 1985; Howard et al. 1997; Petersson et al. 1999; Kangas and Lindstrom 2001; Wong 2004a; Reichard et al. 2005).

Although it is easy to approach the roles of male competition and female choice by studying isolated pairwise interactions, the results of such studies may not be accurate indicators of how sexual selection emerges from interactions in more complex social systems. Male behaviors toward females can be influenced by the presence of other males (e.g., Travis and Woodward 1989; Wong 2004b), male interactions can be altered by the presence of females (e.g., Doutrelant et al. 2001), and female behaviors can be altered by the presence of males and females (e.g., Pilastro et al. 2003).

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Although a number of studies have incorporated both female choice and male competition (reviewed in Wong and Candolin 2005), studies of sexual selection have relied heavily on simple measures of female preference divorced from realistic social interactions. Female dichotomous choice tests are often used to measure female preference in the absence of male interactions. The difficulty is in inferring what these data mean. In fish studies, for example, in some cases, there is a good relationship between female preference and subsequent male reproductive success (e.g., Forsgren 1992; Berglund 1993; Kodric-Brown 1993; Brooks and Endler 2001), but in other cases there is not (e.g., Pyron 1995; Petersson et al. 1999; Fuller 2003; Reichard et al. 2005). Where male interactions play a predominant role, male competition may overwhelm any female preferences (e.g., Petersson et al. 1999; Reichard et al. 2005). In this light, it may not be clear what measures of female preference isolated from social interactions actually imply about the sexual selection process.

In this study, we investigated the interaction between female choice and male competition in the bluefin killifish, *Lucania goodei*, in a 3-staged experiment where 1) females were allowed to choose between 2 males, 2) those males were allowed to compete for a spawning site in the presence of that female, and 3) females and males were allowed to freely interact and spawn in a common environment. Our study addressed 4 questions: 1) Do females exhibit a mate preference? 2) Do males exhibit pairwise dominance relationships? 3) Does female preference predict male dominance? 4) Which factors predict differential spawning success between males?

STUDY SYSTEM

Lucania goodei is a freshwater fundulid found in springs and river drainages of the southeastern United States. Females are silver and cryptic, and males have a conspicuous color polymorphism consisting of various combinations of red, yellow, and blue on their dorsal, anal, and pelvic fins as well as a red spot at the base of the caudal fin. Fertilization is external. Neither sex exhibits parental care, and both sexes engage in filial cannibalism (Fuller and Travis 2001). Although males offer no direct parental care, they aggressively defend areas of aquatic vegetation from other males and spawning occurs in these male territories (Foster 1967; Fuller 2001). Females may visit several male territories each day to spawn and will likely spawn with many different males throughout the reproductive season. Males actively court females by circling females and performing bouts of “head flicks” below the female (Foster 1967). There is no evidence of alternative male mating strategies (McGhee KM, Fuller RC, personal observation).

EXPERIMENTAL METHODS

We collected fish by dipnet and seine from the Wakulla River (Wakulla County, FL) and maintained them in the laboratory for at least 1 week prior to use. We paired females and males randomly and housed each pair in a 10-gallon tank with a yarn mop for shelter and spawning. We fed fish frozen *Artemia* daily and kept them on a 14:10 h light:dark cycle.

To ensure the presence of fresh eggs, we isolated gravid females in 10-gallon tanks 2 days before being used in an experimental trial. The evening before an experimental trial, we randomly chose 2 males and assigned them by coin toss to the left or right chamber of a 30-gallon dichotomous choice tank. We placed an isolated test female in a clear container in the center of the tank. We left the test fish in the tank overnight and began the trials the next morning. There was a yarn mop in each male chamber and a yarn mop in the center of

the main chamber (Supplementary Figure 1a). A single experimental trial consisted of 3 stages.

Stage 1: choice

We investigated female choice without the effect of male–male interactions with dichotomous choice tests where females could visually assess males, but males were kept behind solid transparent plexiglass walls at each end of the tank (Supplementary Figure 1a). Once both males had emerged from their yarn mops, we released the test female. We began videotaping once the female had emerged from her yarn mop. We videotaped each trial for 15 min. From the videos, we calculated the amount of time females spent within 7 cm of each male’s chamber and facing that male. These 7-cm zones next to each male chamber were, together, 32% of the central chamber, whereas the neutral zone was the remaining 68%. The female responded to both males in all trials.

Stage 2: competition

In this stage, we investigated competition between males in the presence of a female but without the possibility of spawning. After completion of Stage 1, we moved the 3 fish immediately to a new 30-gallon tank. We put the female behind a transparent, porous barrier at one end of the tank, and we put the 2 males in the main chamber with 2 yarn mops where they were free to interact (Supplementary Figure 1b). We began the trial once both males emerged from the yarn mops and interacted with each other. We recorded all aggressive behaviors (fin flares, chases, attacks, and circle fights) for 15 min. We determined dominance status based on the outcome of male interactions. For example, we considered a male dominant when the flaring of his dorsal and anal fins and curving of his body toward the second male caused the second male to lower his fins and/or retreat. We also considered a male dominant when he repeatedly attacked the second male and won “circle fights,” which consist of males lining up head to tail and rapidly lunging at each other while moving toward the surface resulting in one male retreating with fins lowered. Although such attacks and fights can cause torn fins and bruising, these trials were of short duration, and no males were critically injured (ACUC approval #0003). These behaviors are not laboratory artifacts and occur throughout the breeding season in nature.

Stage 3: interaction

In the third stage, we let male–male and male–female interactions occur simultaneously. After completion of Stage 2, we released the female and allowed her to interact with the males in the main chamber for 50–55 min (Supplementary Figure 1c). Variation in trial times was due to variation in how long it took the female to leave her chamber and begin swimming freely. In one trial, this stage was accidentally extended to 75 min. We assessed male dominance based on aggressive behaviors as described above and recorded any changes in dominance status from Stage 2 to Stage 3. In addition, we recorded all aggressive behaviors directed toward females (fin flares and attacks), male courtship bouts, and spawning events.

After completion of the 3 stages of a trial, we measured the standard length and wet weight of all fish. We tested each male only once; however, we accidentally reused 4 females. Because all females used in this study were field caught, housed with males in the laboratory, and isolated for 48 h prior to testing, it is unlikely that reuse significantly affected the behavior of these 4 females. In addition, these females were not immediately retested and the time period between

their first and second trials ranged from 7 to 41 days. The results for our analyses on female preference also remain robust after removing these 4 females' second trial from the data set (results not shown).

STATISTICAL METHODS

For any particular female, we know her relative preference for each of the 2 males to which she was exposed in her trial. For any particular pair of males within a trial, we know their dominance status based on the outcome of their aggressive interactions. This design allows us to examine how female preference and male status are influenced by the differences between the 2 males within each trial. We designated each male as 1 or 2 based on the tank chamber he was assigned by coin toss in Stage 1. We then subtracted values for male 2 from the values for male 1. Thus, greater values for male 1 will be positive and greater values for male 2 will be negative. For example, if a female spent more time with male 2, her "preference" score would be negative; if preference was based on male size differences, then the negative score for the preference difference would be associated with a negative score for body size differences. These relative differences can be examined with regression analyses forced through the origin. This method also has the advantage of taking into account continuous variation in the strength of female preference and male behaviors.

Do females exhibit a preference?

We assume that the amount of time a female spends associated with a male in the Stage 1 dichotomous choice test corresponds to the strength of her preference for that particular male, with more time indicating a stronger preference. We examined whether male morphological traits would predict the strength of female preference in Stage 1 by regressing the difference in time spent with each male against the corresponding differences between the males in standard length and body condition, using multiple regression with forward selection and backward elimination to assess consistency of results. The threshold for inclusion in all multiple regression models was $\alpha > 0.05$. We calculated body condition from the residuals of a least-squares linear regression of log-transformed standard length on log-transformed wet weight.

We also examined a subset of all trials ($n = 19$) in which the 2 males were different color morphs (yellow vs. red) to determine whether females preferred males of one color over the other. Because color morph is a categorical variable, we used a *G*-test of independence with a correction for low sample size (Sokal and Rohlf 1995). For this analysis, we categorized males as "preferred" or "nonpreferred" based on the amount of time that females spent associated with each male (i.e., the male with whom she spent more time with was preferred).

Do males exhibit pairwise dominance relationships?

We examined whether male morphological traits and the strength of female preference in Stage 1 could predict male dominance and the amount of aggression in Stage 2 by regressing the difference between the males in the total number of Stage 2 aggressive behaviors directed toward the other male (fin flares + attacks + chases) against the corresponding differences between them in standard length, body condition, and the amount of Stage 1 female association time. Only trials in which dominance was established were used. In these multiple regressions, we used both forward selection and backward elimination to assess the consistency of results. For

trials involving yellow versus red color morphs, we examined whether coloration affected dominance status as described above. For this analysis, we categorized males as "dominant" or "subordinate" based on the outcome of aggressive interactions.

We examined whether male interactions in the presence of a released female (Stage 3) could be predicted by male morphological traits, the strength of female preference (Stage 1), and the male interactions observed in the presence of a restrained female (Stage 2). To do this, we performed a multiple regression in which we regressed the difference between the males of a pair in the total number of Stage 3 aggressive behaviors directed toward the other male (fin flares + attacks + chases) against the corresponding differences between them in standard length, body condition, the amount of Stage 1 female association time, and the amount of Stage 2 aggression toward males (fin flares + attacks + chases) in trials where dominance was established. As described above, we used both forward selection and backward elimination and examined whether coloration affected dominance status in trials involving yellow versus red color morphs.

To examine the relative number of behaviors performed by males of a pair in Stage 3, we examined the proportion of behaviors performed by male 1 using Spearman rank correlations on the untransformed proportions. In this way, we can determine whether behavioral activity is skewed toward one male in the pair or whether both males of a pair perform an equal proportion of the behaviors.

We also examined whether there were correlations in the rates at which individual behaviors were expressed by calculating the Spearman rank correlations among all behaviors expressed by the dominant male of the trial pair. To be specific, we examined whether courtship and aggressive behaviors toward both females and males are correlated.

We had initially intended to score all behaviors except for the number of spawns as presence/absence and did not begin recording the numbers of behaviors until after the first 10 trials. Therefore, the sample sizes in the analyses examining the number of aggressive behaviors are lower than those for other analyses ($n = 23, 24$).

Which factors predict differential spawning success between males?

The ultimate measure of male mating success in this system is the number of spawns that a male obtains from a female. We examined whether differences between males in the number of spawns could be predicted by differences in female preference and differences in a variety of male traits. All the Stage 3 male behaviors (aggression toward males, aggression toward females, and courtship) were significantly correlated with one another, so we used principal components (PCs) analysis to get composite scores of these Stage 3 behaviors (Table 1). The first PC explains 74% of the variation in the Stage 3 behaviors and indicates an individual's overall activity level, with positive values corresponding to increasing aggression to both males and females as well as increasing courtship behavior (Table 1). The second PC explains an additional 17% of the variation in the Stage 3 behaviors and indicates an individual's behavior toward females, with positive values corresponding to increasing aggression to the female and decreasing courtship behavior (Table 1). We then used these PC scores as predictors in the regression analysis analogous to using morphological and other traits. For example, in each trial, we subtracted the PC1 score for male 1 from the PC1 score for male 2 to obtain the difference between males within a pair for this particular composite score of the Stage 3 behaviors. Thus, in our multiple regression, we regressed the differences between males of

Table 1
The structure coefficients for the principal components

	PC1	PC2
Number of aggressive behaviors toward males	0.904**	-0.164
Number of aggressive behaviors toward female	0.800**	0.592**
Number of courtship bouts	0.869**	-0.374*
Cumulative variance explained	0.737	0.910

* Indicates significant correlations at $\alpha = 0.01$.

** Indicates significant correlations at $\alpha < 0.0001$.

a pair in the number of spawns they obtained in Stage 3 against their differences in standard length, body condition, the amount of Stage 1 female association time, the amount of Stage 2 aggression toward males (fin flares + attacks + chases) in trials where dominance was established, the amount of Stage 3 behavioral activity (PC1), and the type of Stage 3 behaviors directed toward females (courtship vs. aggression) (PC2).

Finally, we investigated whether females exercise mate choice by adjusting how quickly they spawn with a particular male. To do this, we examined the relationship between a female’s Stage 1 association time and her latency to spawn with that male in Stage 3 using Pearson correlation analyses on log-transformed data. We also examined whether females adjusted the number of eggs they released with their Stage 1 preferred and nonpreferred males using paired *t*-tests.

Multiple tests

In our analyses, we report the unadjusted *P* values. To reduce the probability of committing a Type 1 error by performing multiple statistical tests, we used the Dunn-Sidak method (Sokal and Rohlf 1995), where $\alpha' = 1 - (1 - \alpha)^{1/k}$, where *k* equals the number of tests performed. We performed all analysis using SAS software, version 9.1.

Table 2
Summary of stepwise multiple regression results

Dependent variable (male 1–male 2)	Independent variables ^a (male 1–male 2)	<i>F</i>	<i>P</i> ^b	<i>R</i> ² value ^c
Stage 1: female association time (preference)	Standard length	0.02	0.878	0.007
	Body condition	0.20	0.655	0.006
Stage 2: aggression toward other male (dominance)	Standard length	0.44	0.519	0.143
	Body condition	0.89	0.359	0.118
	Stage 1: female association time	1.26	0.277	0.069
Stage 3: aggression toward other male (dominance)	Body condition	0.20	0.665	0.479
	Standard length	3.27	0.091	0.472
	Stage 1: female association time	3.20	0.093	0.357
	Stage 2: male aggression	5.04	0.038	0.229
Number of spawns	Body condition	0.03	0.875	0.672
	Standard length	0.05	0.828	0.672
	Stage 3: male behavior toward female (PC2)	0.09	0.774	0.670
	Stage 1: female association time	0.44	0.519	0.668
	Stage 2: male aggression	2.83	0.112	0.659
	Stage 3: male activity level (PC1)	25.32	0.0001	0.598

^a Predictors are arranged in the order in which they were eliminated from the model.

^b Variables in bold are significant at $\alpha = 0.05$ and were retained in the model.

^c *R*² values show the variance in the dependent variable that is accounted for by that independent variable and all the remaining variables in the model listed below it.

RESULTS

We restricted all our analyses to trials in which the female spawned (34 of 45 trials). Trials without spawning may be due to a lack of mature eggs (5/11 trials) or an inability to make a spawning choice within the trial time period for some unknown reason despite the presence of mature eggs (6/11 trials). In the 6 trials where the female had mature eggs but did not spawn, the female preferred the future Stage 3 dominant male in 4 trials and the subordinate male in 1 trial. There were no male interactions in 1 trial.

Do females exhibit a preference?

Every female spent substantially more time with one of the males in the dichotomous choice test. The average time spent with her preferred male was 5.67 ± 0.54 min (average \pm standard error), whereas the average time spent with her non-preferred male was 1.44 ± 0.22 min. The average difference between time spent with each male is significantly different from zero (degrees of freedom [df] = 33, $t = 6.957$, $P < 0.0001$). On average, females spent about 53% of the time in the “neutral zone” or not facing a male (7.89 ± 0.56 min), which means that, on average, a female spent 72% of the remaining time with one particular male.

We found no consistent differences between the male preferred by females and the one that was not. Neither the difference between males in standard length or body condition significantly explained the difference between males of a pair in Stage 1 female association time (Table 2). In those trials where the female was given a choice between red and yellow males, she did not prefer one color over the other consistently ($n = 19$, $G_{adj} = 0.463$, $P = 0.496$). The color of the male with which she was housed prior to testing also did not affect her preference ($n = 22$, $G_{adj} = 0.713$, $P = 0.398$).

Do males exhibit pairwise dominance relationships?

In Stage 2, one male established dominance over the other in 27 out of the 34 trials (the remaining 7 trials were excluded from further analysis of Stage 2 results). In these 27 trials, the difference between males of a pair in the amount of Stage 2

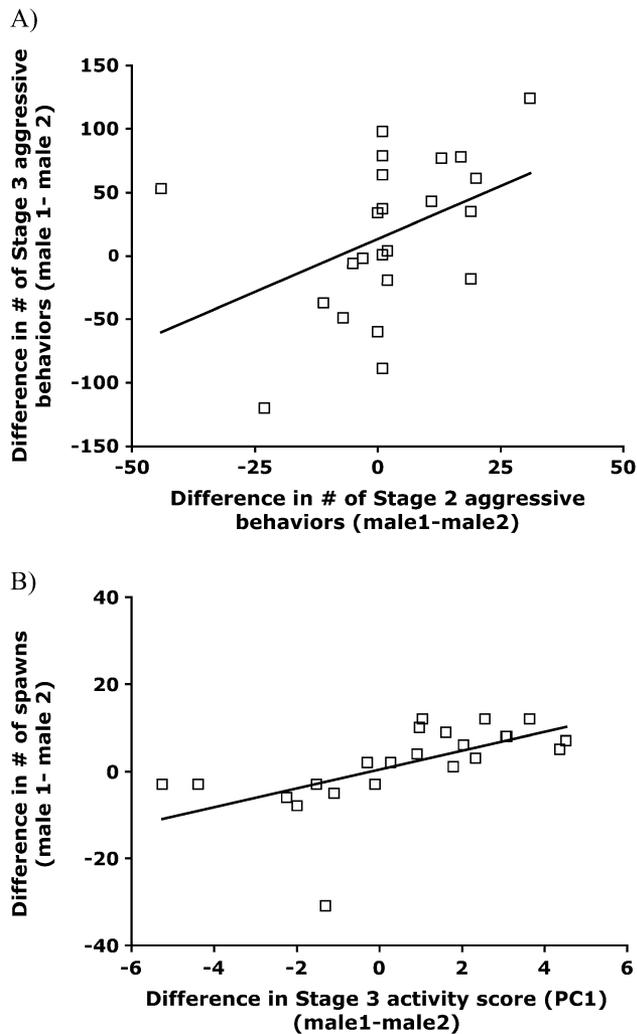


Figure 1
The relationship between (A) the difference between the males in a pair in the number of aggressive behaviors exhibited towards the other male in Stage 3 and the difference between them in the number of aggressive behaviors exhibited towards the other male in Stage 2, and (B) the difference between the males in a pair in the number of spawns they obtained in Stage 3 and the difference between them in their Stage 3 activity score (PC1). Note that regression lines are forced through the origin.

aggression directed toward the other male was not significantly related to the difference between them in standard length or body condition (Table 2) nor did female preference predict Stage 2 male aggression (Table 2). In those trials where the males were of different colors, male color was not associated with Stage 2 male dominance ($n = 16$, $G_{\text{adj}} = 0$, $P = 1.0$).

In Stage 3, dominance was established in all 34 trials. In 20% of the trials (7/34), male dominance status switched from Stage 2 to Stage 3 with the initially inactive subordinate male becoming dominant once the female was released. Free interactions with the female in Stage 3 caused the aggressive behaviors seen in Stage 2 to be exaggerated resulting in the elimination of ambiguity in dominance relationships. Despite this dominance “switching” in 7 trials and the fact that dominance was not established in an additional 7 trials in Stage 2, the difference between males in Stage 2 aggression predicted the difference between the males in Stage 3 aggression; the male that was more aggressive in Stage 2 tended to be more aggressive in Stage 3 (Figure 1A, Table 2). This difference in

Stage 2 aggression accounted for 23% of the variance in the difference in Stage 3 aggression (Table 2). There was no effect of the differences between males in standard length or body condition in Stage 3 aggression (Table 2). In those trials where males were of different colors, male color was not associated with Stage 3 male dominance ($n = 19$, $G_{\text{adj}} = 0.463$, $P = 0.496$).

The expression of behaviors was skewed toward one male, as evidenced by the high correlations among the proportions of behaviors exhibited (Table 3). Within a pair, one male emerged as dominant and performed most, if not all, of the aggressive behaviors toward both males and females, as well as most, if not all, of the courtship bouts. Dominant males who expressed higher rates of one behavior also expressed higher rates of other behaviors; there were positive correlations among the suite of aggressive behaviors toward other males, aggressive behaviors exhibited toward females, and the number of courtship bouts they performed (Table 4, Figure 2).

Which factors predict differential spawning success between males?

In the majority of trials (29/34), the female spawned exclusively with the dominant male (Table 3). In the 5 trials where the female spawned with both the subordinate and dominant male of the pair, the first male the female spawned with upon release tried to establish dominance but failed and reverted to a subordinate role. Once the dominant male spawned with the female, the subordinate male received no further spawning opportunities. The number of spawns obtained by a male in a trial ranged from 0 to 31.

The differences between males in the number of spawns they acquired was predicted quite well by the differences in their “overall activity” scores (PC1), a prediction that accounted for 60% of the variance in the difference in number of spawns (Figure 1B, Table 2). The more active and aggressive a male was in the trial, the more spawns he obtained. No other factor predicted differences between males in the number of spawns they acquired. Although the measure of female preference in Stage 1 did not predict dominance status or spawning success, it did reveal something about female predilections; females spawned more quickly with the male with whom they had spent the most time (Figure 3). The correlation between time associated with a particular male in Stage 1 and latency to spawn with him in Stage 3 was -0.72 ($n = 27$, $P < 0.0001$). The total number of eggs a female released during a trial was highly variable (range = 1–43 eggs). As one would predict based on trial time constraints, females who spawned quickly also spawned more often and released more eggs (correlation between the number of eggs spawned and the latency to spawn in Stage 3 was -0.62 , $n = 20$, $P = 0.0037$). The total number of eggs a female released did not differ between her Stage 1 preferred and nonpreferred males ($df = 27$, $t = -0.015$, $P = 0.989$; trials in which both males spawned were excluded). A female’s standard length was not correlated with the total number of eggs she released in a trial ($n = 34$, $r = 0.21$, $P = 0.229$).

DISCUSSION

In this experiment, male dominance was critical in determining mating success (Table 3). Interference from dominant males kept subordinates from courting or spawning with females. These patterns are consistent with field observations of *L. goodei* where male aggression is a strong correlate of courtship behavior and spawning success (Fuller 2001).

Although female preferences did not predict male dominance status or the number of spawns, it is incorrect to infer

Table 3
Spearman rank correlation coefficients between the proportion of behaviors performed by male 1 toward males and females

		Toward males ^a			Toward females ^a			
		No. fin flares	No. attacks	No. chases	No. fin flares	No. attacks	No. courtship bouts	No. spawns
Toward males	No. fin flares	—	0.945 (22) <0.0001	0.942 (19) <0.0001	0.850 (23) <0.0001	0.860 (16) <0.0001	0.856 (24) <0.0001	0.918 (24) <0.0001
	No. attacks		—	1.000 (19) <0.0001	0.925 (21) <0.0001	0.932 (15) <0.0001	0.913 (23) <0.0001	0.967 (22) <0.0001
	No. chases			—	0.979 (19) <0.0001	0.932 (15) <0.0001	0.946 (19) <0.0001	1.000 (19) <0.0001
Toward females	No. fin flares				—	0.869 (16) <0.0001	0.902 (23) <0.0001	0.907 (23) <0.0001
	No. attacks					—	0.990 (16) <0.0001	0.932 (16) <0.0001
	No. courtship bouts						—	1.000 (24) <0.0001

P values in bold are significant after correcting for multiple tests using the Dunn-Šidák method ($k = 21, \alpha = 0.0024$).

^a Values are Spearman correlation coefficients (r) with samples sizes in parentheses. *P* values are listed below the Spearman correlation coefficients.

that female mating preferences are unimportant in *L. goodei*. Females spawned more quickly when their preferred male emerged as dominant, and they took longer to spawn when their nonpreferred male emerged as dominant (Figure 3). It is crucial to keep in mind that, in addition to controlling the latency to spawn, females can spawn multiple times in quick succession and release a variable number of eggs at each spawn. This control means that female mating preferences can influence the absolute fitness of an individual male, the relative fitness of different males, and therefore the strength of sexual selection.

How do we reconcile the expression of female mating preferences with the emergence of male dominance? Males are territorial and dominant males seem more likely to acquire and retain territories. In nature, females circulate amongst male territories and spawn with numerous males over the course of the spawning season. We suspect that females are choosing among the many males that are defending territo-

ries, moving from one “preferred” dominant male’s territory to another. In this scenario, interactions among males determine the initial mating pool, whereas females exert mate choice amongst dominant territory-holding males by controlling the number of visits, the latency to spawn, and the number of eggs released, thereby determining the extent of any particular male’s mating success.

There is evidence in our results for 2 potential forms of sexual conflict. First, there is some conflict over the outcome of mating. In almost half of the trials (16/34), males preferred by the female did not emerge as dominant and most did not spawn (12/34). Although we do not know which aspects of the male’s phenotype are under selection (see below), this pattern indicates that there are clear conflicts of interest between males and females in some cases. It should be noted that visual contact between the males in the Stage 1 dichotomous choice tank may have affected Stage 1 male behaviors and, thus, female preference (e.g., Kangas and Lindström 2001).

Table 4
Spearman rank correlation coefficients between the number of different behaviors performed by the dominant male of a pair

		Toward males ^a			Toward females ^a		
		No. fin flares	No. attacks	No. chases	No. fin flares	No. attacks	No. courtship bouts
Toward males	No. fin flares	—	0.753 (24) <0.0001*	0.444 (23) 0.0336	0.552 (23) 0.0063	0.350 (23) 0.1014	0.517 (24) 0.0097
	No. attacks		—	0.671 (23) 0.0005*	0.401 (23) 0.0579	0.614 (23) 0.0018*	0.548 (24) 0.0056
	No. chases			—	0.232 (23) 0.2871	0.329 (23) 0.1255	0.536 (23) 0.0084
Toward females	No. fin flares				—	0.494 (23) 0.0166	0.273 (23) 0.2080
	No. attacks					—	0.414 (23) 0.0495

P values in bold are significant at $\alpha = 0.05$. “*” Indicates correlations that are significant after correcting for multiple tests using the Dunn-Šidák method ($k = 15, \alpha = 0.0034$).

^a Values are Spearman correlation coefficients (r) with samples sizes in parentheses. *P* values are listed below the Spearman correlation coefficients.

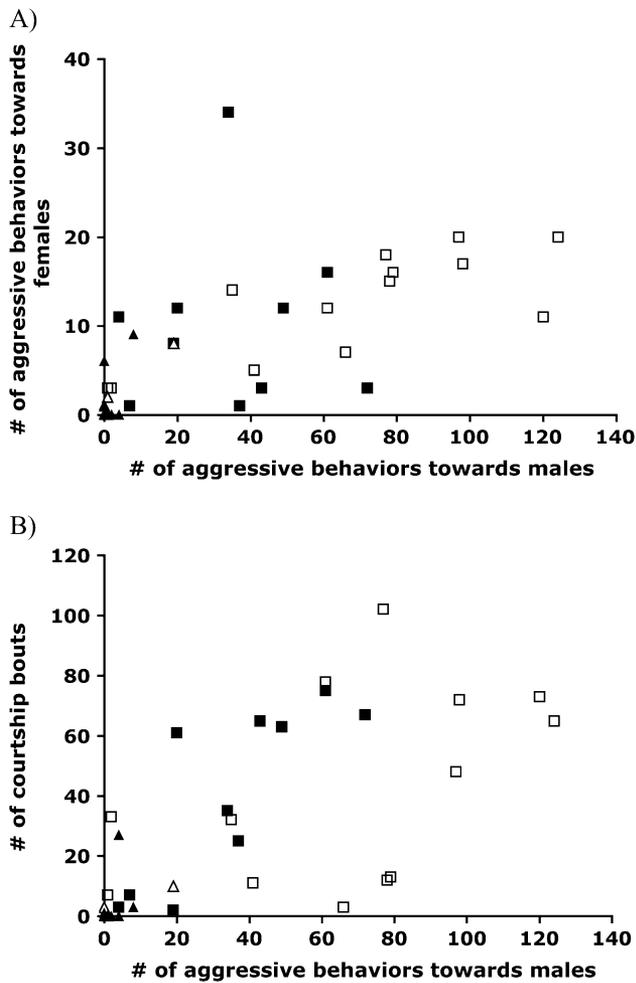


Figure 2

The relationship between dominant and subordinate males in (A) the total number of aggressive behaviors towards the other male (sum of fin flares, attacks, and chases) and the total number of aggressive behaviors towards the female (sum of fin flares and attacks) ($n = 23$), and (B) the total number of aggressive behaviors towards the other male (sum of fin flares, attacks, and chases) and the total number of courtship bouts ($n = 24$). Open square = Stage 1 preferred, Stage 3 dominant males; filled square = Stage 1 nonpreferred, Stage 3 dominant males; open triangle = Stage 1 preferred, Stage 3 subordinate males; filled triangle = Stage 1 nonpreferred, Stage 3 subordinate males.

Although we cannot eliminate this possibility due to our tank design, no male aggressive behaviors were observed in Stage 1 and courtship displays did not seem elevated compared with the single males in housing tanks. It is also possible that chemical cues are involved in female preference (de Caprona and Ryan 1990; Hankison and Morris 2003) and that the relationship between female preference and male dominance was affected by the prevention of chemical communication in the Stage 1 dichotomous choice tank. Unfortunately, the role of chemical signals in *L. goodei* interactions remains unknown.

Second, there is evidence for a direct cost to females who associate with dominant males for an extended period of time. Males who were more aggressive to other males and became dominant were also more aggressive toward females (Figure 2A). This is not an artifact of measuring behavior in the laboratory. Fuller (2001) has also demonstrated that males frequently attack conspecific females as well as heterospecifics (usually minnows) in the field. Female choice, as measured by

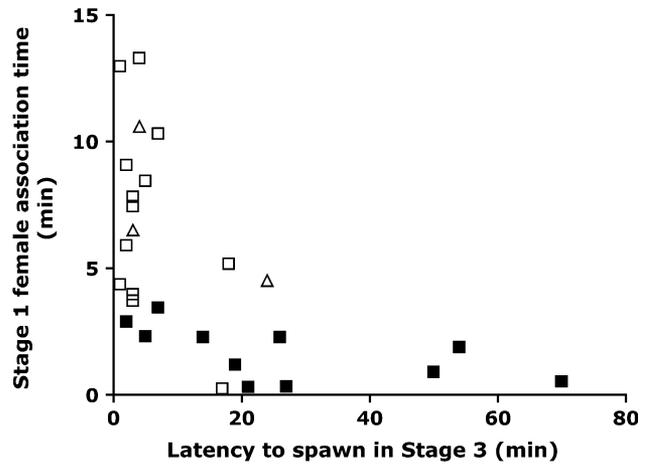


Figure 3

The correlation between the amount of time a female spent associated with a particular male in Stage 1 and her latency to spawn with that male in Stage 3 ($n = 27$, 3 trials are included where both the dominant and subordinate male spawned). Analysis was on log-transformed data. Open square = Stage 1 preferred, Stage 3 dominant males; filled square = Stage 1 nonpreferred, Stage 3 dominant males; open triangle = Stage 1 preferred, Stage 3 subordinate males.

latency to spawn, may act to reduce the amount of time spent with a dominant aggressive male, and indeed, we found that females spawned more quickly with dominant males that they had preferred in Stage 1 (Figure 3).

Despite clear evidence for the importance of male competition (and to a lesser extent, female mating preferences), none of the male morphological traits we measured, including color, were associated with either female preference or male dominance (Table 2). This is in contrast to a number of studies that have found female preference for male size (e.g., Ptacek and Travis 1997; Rosenthal and Evans 1998; MacLaren et al. 2004; Reichard et al. 2005; but see Forsgren 1997; Wong 2004b, Shackleton et al. 2005) and aspects of male coloration (e.g., Kodric-Brown 1985; Hill 1990; Milinski and Bakker 1990; Wong 2004b). This is also in contrast to a number of studies that have found male size to be a good predictor of dominance (e.g., Forsgren 1997; McElligott et al. 2001; Wong 2004b; Reichard et al. 2005; Shackleton et al. 2005; Fisher and Cockburn 2006) and studies that have demonstrated the role of male color polymorphisms in aggressive interactions (e.g., Sinervo and Lively 1996; Pryke and Griffith 2006). Consistent with our findings on male coloration, previous studies on *L. goodei* have found aggression (Fuller and Travis 2001) and fertilization success (Fuller 2001; Fuller and Travis 2001) do not vary with color morph both in the field and the laboratory. Although the frequencies of the different *L. goodei* color morphs vary across signaling environment (Fuller 2002), as do aspects of the visual system (Fuller et al. 2003; Fuller et al. 2004), what role color morph plays in signaling and the evolutionary processes maintaining multiple color morphs (many of which have a genetic basis; Fuller and Travis 2004) in natural populations remains unknown. In terms of the traits preferred by females, it is important to keep in mind that unlike species where females prefer traits that increase egg hatching success, such as good paternal care (e.g., Forsgren 1997; Candolin 2000; Wong 2004b), *L. goodei* does not provide any care to eggs and engages in filial cannibalism (Fuller and Travis 2001) although it is possible that territory defense affects egg survival indirectly.

Although we were unable to identify morphological correlates of mating success, we clearly identified behavioral

correlates of mating success where the relative amount of aggressive behavior a male of a pair exhibits toward the other male was the most important variable in determining spawning success (Tables 2 and 3). It is generally assumed that many traits used as cues in sexual selection are likely to be condition dependent (Rowe and Houle 1996; Cotton et al. 2004). It is possible that females and males may be assessing male quality based on condition-dependent behaviors rather than condition-dependent morphological traits. Dominant males may be those males that are in good condition regardless of size (Royle et al. 2005), and as a result, they are better able to mount aggressive attacks and defend a territory from other males. We found that dominant and subordinate males are easily distinguishable by a variety of continuously varying behaviors and represent different behavioral phenotypes. Males may be exhibiting different behavioral syndromes (Sih, Bell, and Johnson 2004, Sih, Bell, et al. 2004) with some individuals showing an overall elevated level of behavior and others showing a reduced level of behavior. Before any conclusions can be drawn, however, we must distinguish between whether dominance status is an attribute of the individual or the attribute of the particular dyad or social group. One of us (K.E.M.) is presently investigating this, as well as the role of social environment and nutrition on future dominance behavior and female preference. The extent to which these different behaviors are genetically correlated will shed light on the evolution of the suites of behavior used in both male–male and female–male interactions and how they affect spawning success.

Interestingly, male dominance switched between Stage 2 and Stage 3 in 20% of the trials, and in 20% of Stage 2 trials, male dominance was not even established. This finding emphasizes the necessity of allowing free social interactions in their entirety rather than using isolated behaviors or partial interactions as proxies to understand the sexual selection process. It seems that, at least in *L. goodei*, the visual and chemical presence of a female is not always adequate to induce male struggles over dominance. Males of some species may require actual behavioral interaction with females to assess the potential of a mating opportunity and initiate male competition (e.g., Sumner et al. 1994). Tiered experiments, such as ours and those of others (e.g., Petersson et al. 1999; Candolin 2004), allow a variety of social interactions to occur and may be extremely useful in determining the relative roles of female choice, male–male competition, and their interaction on mating success, ultimately leading to a better understanding of the outcome of sexual selection in nature.

SUPPLEMENTARY MATERIAL

Supplementary Figure 1 can be found at <http://www.behco.oxfordjournals.org/>

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