Intra- and intersexual selection on male body size are complimentary in the fathead minnow (*Pimephales promelas*)

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Summary

Intrasexual competition between males and intersexual mate choice by females may have complementary or antagonistic effects on the evolution of sexually-selected traits. We used open-aquarium experiments to test for the effects of male body size and male secondary sexual characters on male–male competition and on female mate choice in the fathead minnow *Pimephales promelas*. Larger males were more successful than smaller males in competing for nesting substrates. Larger males were also preferentially chosen by females for spawning. Secondary sexual characters (tubercle number, banding pattern, and dorsal pad development) were not associated with success in male–male contests and were not preferred by females. In a separate experiment, we found that female choice was not influenced by past male reproductive success. We confirmed genetic paternity of fertilized eggs with microsatellites and showed that our results were not complicated by clutch stealing or multiple paternity. Collectively, our results suggest that male–male competition and female mate choice have complimentary effects on the evolution of large male body size in fathead minnows.

Keywords: sexual selection, *Pimephales promelas*, fathead minnow.

Introduction

Sexual selection is primarily driven by interactions between individuals of the same sex (intrasexual competition for access to mates) and interactions between individuals of opposite sexes (intersexual mate choice; Darwin,

1871; Andersson, 1994). An underlying assumption in sexual selection research has been that intra- and intersexual processes of sexual selection are complementary: the trait or suite of traits that determines the outcome of intrasexual competition accurately conveys the quality of potential suitors during intersexual mate choice (Candolin, 1999; Berglund & Rosenqvist, 2001). Indeed, success during intrasexual competition can be indicative of a suitor's quality (Bisazza et al., 1989; Montgomerie & Thornhill, 1989; Alatalo et al., 1991; Kodric-Brown, 1996), and there is empirical evidence that both intra- and intersexual processes may favour the same phenotypes (e.g., Berglund et al., 1986; Milinski & Bakker, 1990; De Fraipont et al., 1993; Rosenthal & Evans, 1998; Aspbury & Basolo, 2002; Basolo, 2004). Nevertheless, this assumption is usually not tested, and recent focus has been on understanding the relative strength of and interactions between the intra- and intersexual processes underlying sexual selection (Qvarnstrom & Forsgren, 1998; Moore & Moore, 1999; Bonduriansky & Rowe, 2003; Maynard Smith & Harper, 2003; Candolin, 2004; Wong & Candolin, 2005).

In some mating systems, intrasexual competition facilitates the outcome of intersexual mate choice (e.g., Candolin, 1999), but in some cases the processes are antagonistic (Bernet et al., 1998; Berglund & Rosenqvist, 2001). Moreover, sexual conflict theory (Parker, 1979) suggests that mate quality and dominance status are not always positively correlated. For example, males adept at winning intra-sexual contests may increase their own mating opportunities by excluding rivals, but females may, in turn, have reduced fitness when mating with dominant males (Forsgren, 1997; Chapman, 2001; Chippindale et al., 2001; Wong, 2004). Accordingly, females may discriminate against dominant males in favour of subordinates (e.g., Moore & Moore, 1999) to increase their own fitness.

During mating, both males and females attempt to balance the trade-off between the costs of mating and the fitness benefits gained from employing a certain strategy, and the balance is not necessarily complementary. Teasing apart the influences of intrasexual and intersexual components of sexual selection is difficult, however, because males and females interact and respond simultaneously to one another. Experimental studies that allow for separate measures of male–male competition and female choice in the same individuals can overcome this limitation. For this study, we used an open aquarium design (Houde, 1997) to assess the effects of male body size, secondary

sex characters, and behaviour on male–male competition for nesting substrates and female mate choice in the fathead minnow (*Pimephales promelas*). Male–male competition and female preference both favoured large male body size, suggesting that these mechanisms of sexual selection are complimentary with respect to male body size.

Methods

Study system

The fathead minnow is a freshwater cyprinid that inhabits lakes, ponds, and slow-moving streams (Andrews, 1970; Lee et al., 1980). Its geographic range is from southern Canada through the entire continental United States and into northern Mexico (Andrews, 1970; Lee et al., 1980). The sexes are indistinguishable as juveniles, but considerable sexual dimorphism is evident in adults approximately 30 days before spawning begins (Flickinger, 1969). Males develop breeding tubercles (horny projections on the snout and lower jaw), a pad of thickened, mucous-secreting epidermal cells between the head and the dorsal fin, and a contrasting pattern of alternating dark and light bands (Isaak, 1961; McMillan & Smith, 1974; Unger, 1983). Sexually mature males move into shallow water and compete to defend nesting territories established on the undersides of rocks or in stable vegetation (Markus, 1934; Isaak, 1961; Andrews, 1970; McMillan & Smith, 1974). Nesting territories are vigorously defended from other males, as well as from non-gravid females, juveniles, and other potential egg predators (Markus, 1934; Unger, 1983; Pyron & Beitinger, 1989; Sargent, 1989). Males confront nest intruders (egg predators and other males) either with a behavioural display or by butting them with their tubercles. Mature females move singly or in small groups through areas where males have established territories to make spawning decisions. Territorial male fathead minnows court females with a behavioural repertoire that includes approaches, lateral displays, and leading behaviours (Cole & Smith, 1987). Females enter the nests of territorial males and deposit their eggs on the ceiling of the nest cavity (Markus, 1934; Isaak, 1961; Andrews, 1970). Males fertilize and subsequently care for the eggs until they hatch.

Laboratory conditions

Fathead minnows used in this experiment were obtained from Aquatic Research Organisms (Hampton, NH, USA). The fish were housed in 114-l stock tanks until they could be sexed based on external morphology (Flickinger, 1969). Males and females were then separated and housed in 76-l aquaria. Aquaria were maintained at an ambient water temperature of $23 \pm 2°C$ and a 16 h/8 h (light/dark) photoperiod to maintain adults in reproductive condition (Denny, 1987). All fish were fed twice daily using flake food or frozen brine shrimp. This project was carried out in accordance with the methods described in protocols 01-105 and 04-126 approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Vermont.

Experiment 1. Sexual selection during initial spawning

We used a paired contest, open-aquarium design (Houde, 1997) to determine how body size (standard length, SL; wet mass, WM), secondary sex characters (banding pattern, BP; tubercle number, TN; dorsal pad development, DP), and courtship behaviour related to male success during both intrasexual contests for nest sites and intersexual mate choice. Each replicate was divided into three stages: preparation/acclimation, male–male competition, and female choice. Our experiment was initiated with $N = 60$ dyads (=120 males) in the preparation/acclimation stage. Some males, however, did not maintain territoriality throughout the acclimation period, so the sample size for the male–male competition phase of the experiment was $N = 50$ dyads (=100 males). Following the male–male competition phase, some males did not maintain territoriality ($N = 9$), some female did not make a choice in the allotted time ($N = 5$), and there was some mortality ($N = 2$), so the sample size for the female preference experiment was $N = 34$ dyads (=68 males, 34 females).

Preparation/Acclimation ($N = 60$ dyads)

During the preparation/acclimation stage, we randomly selected males beginning to express secondary sex characters from our single-sex stock population and housed them in randomly assigned pairs in 38 l preparation aquaria (Figure 1A). Each aquarium contained three mature females and two nest substrates (10 cm section of 7.6 cm diameter PVC pipe cut in half longitudinally). The five fish were allowed to freely interact until spawning occurred
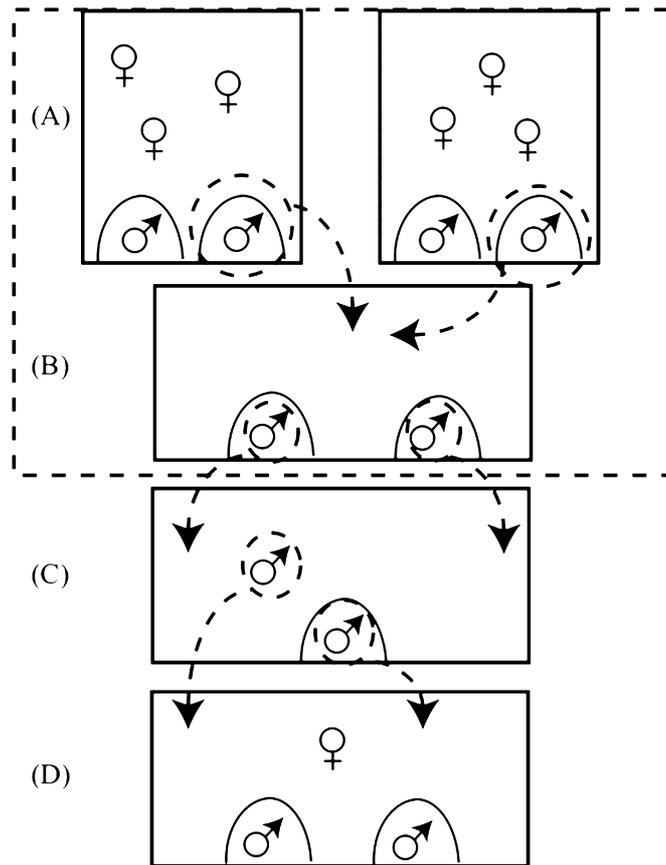
Figure 1. Experimental design and tank arrangement used for Experiment 1. Males were transferred from stock aquaria to 38-l preparation tanks and allowed to interact with three sexually mature females until one member of the pair spawned successfully. When two males were simultaneously guarding eggs in separate preparation tanks (A), they were transferred to an experimental tank and allowed to acclimate (B). Using dyadic contests, we monitored success during intrasexual interactions (C). Using open choice spawning trials we monitored spawning success (D).

and one male was found guarding eggs. To establish dyads for our experiment, we randomly paired two reproductively successful males from separate preparation tanks. Each male was anesthetized using MS-222 (0.3 g/l), measured for standard length and wet mass, and marked for individual identification by clipping a small portion of either the top or bottom lobe of the caudal fin. Both males were placed in a 1-litre chamber until they fully recov-

ered from anaesthesia (approx. 30 min). Once both males had recovered, we released them into a 76-l aquarium containing two identical nest substrates and allowed them to acclimate for approx. 12 h (Figure 1B).

Male–male competition ($N = 50$ dyads)

Following the acclimation period, we scored the banding pattern of each male on a scale of 1 (=lowest intensity) to 4 (highest intensity; Unger, 1983; see also Danylchuk & Tonn, 2001), removed the two nest substrates, and replaced them with a single fresh substrate in the centre of the tank (Figure 1C). After the introduction of the new nest substrate, we quantified the number of seconds each male spent guarding the nest over a 15-min. observation period (=resource holding power, RHP). All replicates resulted in a clear winner.

Female choice ($N = 34$ dyads)

During the female choice stage, we removed the central nest from the tank containing the two males and replaced it with two new nests (Figure 1D). We then introduced a gravid virgin female (unfamiliar to both males) in the tank and allowed her to swim freely and acclimate for approx. 8 h. We quantified male–female behavioural interactions by videotaping 17 randomly selected replicates for 10 min during the morning (0800-1000) and during the evening (1600-1800) each day until spawning occurred. We later scored the video-taped interactions for the number of courtship approaches the male made toward the female (Cole & Smith, 1987; Vives, 1988) and the amount of time the male spent guarding his nest. Males and females included in this stage of the experiment were allowed to freely interact until spawning occurred. Following the observation period, we captured both males, anesthetized them, measured standard length and wet mass, and counted their number of tubercles.

Experiment 2. Male reproductive history and female choice

We conducted a second experiment to determine if female preference for a male was influenced by his past reproductive success. As in Experiment 1, we divided the experiment into stages: (1) preparation and (2) female choice.

Preparation ($N = 20$ dyads)

During the preparation stage, we selected two similarly-sized virgin males from a 114-l single-sex stock tank. Each male was anaesthetized and measured as in Experiment 1. We alternately assigned the larger of the two males to mated and unmated treatments ($N = 9$ larger males and $N = 11$ smaller males mated; sample sizes were initially equal, but one or both males died in two replicates in which the larger male was assigned to the mated treatment). This design ensured that the average size difference between males in the mated and unmated treatments did not differ significantly from 0 (matched pairs analysis: mean difference in standard length $= -0.804$ mm, $t = -1.28$, df $= 19$, $p = 0.22$; mean difference in wet mass $= -0.002$ g, $t = -0.02$, df $= 19$, $p = 0.98$), thus allowing us to detect subtle effects of size on male mating success. After measuring the males, we released them into a 1-l chamber to recover from anaesthesia for 30 minutes and then transferred them into their respective 38-l aquaria for the duration of the preparation stage. Mated males were then released into an aquarium containing a single nest substrate and three sexually mature female fathead minnows (Figure 2A). Unmated males were released into a 38-l aquarium containing only a nest substrate (Figure 2B). The preparation period continued until the male in the mated treatment had spawned successfully and was defending eggs.

Female choice ($N = 20$ dyads)

During the female choice stage of the experiment (Figure 2C), the mated and unmated males were both transferred to a 76-l aquarium containing two empty nest substrates and one gravid virgin female fathead minnow. The female choice stage continued until the female had spawned with one of the two males.

Statistical analyses

Because the measurements of SL and WM were highly correlated (Experiment 1: $N = 120$ males, $r = 0.96$, $p < 0.0001$; Experiment 2: $N = 40$ males, $r = 0.89$, $p < 0.0001$), we used only SL as a proxy for body size in our analyses.

Experiment 1. Sexual selection during initial spawning

Male–male competition. — We used multiple linear regression to determine whether body size or secondary sexual characteristics predicted the out-

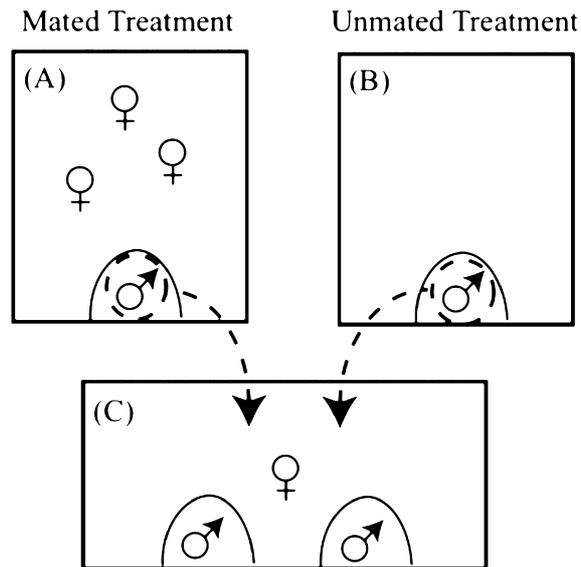Mated Treatment   Unmated Treatment

Figure 2. Experimental design for examining the relationship between male reproductive history and female preference. Large and small males were alternately assigned to the mated or unmated treatments. Mated males were housed in a 38-l aquarium containing a nest substrate and three adult females until at least one female had spawned (A) whereas unmated males were housed alone with a nest substrate (B). Upon successful spawning, both males were transferred to a 76-l aquarium containing a gravid virgin female and two new nest substrates (C). The trial continued until the female elected to spawn with one of the males.

come of male–male contests over a single nest site. Because of the matched-pairs nature of our design, we used the difference (δ) between males for both the predictor (SL, BP, DP, TN) and response (time guarding the nest) variables in our analyses. The predictor and response variables for each dyad were calculated by subtracting the values for the male marked on the bottom of the caudal (B-marked male) fin from the values of the male marked on he top of the caudal fin (T-marked male; e.g., [Top-marked male SL] − [Bottom-marked male SL] = δSL). Our regression model tested the null hypothesis of no association between body size or secondary sexual characters and time spent guarding the nest and, thus, success at male–male combat and social dominance.

Female choice. —   We used a two-tailed binomial test to determine if female fathead minnows preferentially mated with males that had won their

contests over a single nest site. We compared the frequency of winning males that were chosen by females to a null expectation of 0.50.

We used matched-pairs analysis to determine whether body size, or secondary sex characters were associated with the outcome of female choice trials. For each predictor variable, we tested the null hypothesis that the mean difference between chosen and rejected males would be 0. We used the Dunn-Sidak method of stepwise Bonferonni correction (Sokal & Rohlf, 1995) to set the experimentwise $\alpha = 0.05$. Uncorrected p-values are reported in the text with results that were significant after Dunn-Sidak correction indicated by an asterisk (*).

Experiment 2. Male reproductive history and female choice

We tested the hypothesis that male reproductive success in a current mating event was independent of reproductive history by counting the number of previously successful males that were chosen and rejected by females in our experiment. We compared the observed frequencies to our null expectation that females would mate randomly with respect to male reproductive history using a likelihood ratio test.

Paternity analysis

Because male fathead minnows that are not guarding eggs may usurp the nests of males that are guarding (Unger, 1983; Sargent, 1988; Unger & Sargent, 1988), we used microsatellites to confirm the genetic paternity of the eggs spawned during the female choice trials in Experiment 1 and, therefore, eliminate the possibility that the putative father had stolen the clutch from the genetic father.

We used the extraction protocol for animal tissues from a DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) to isolate DNA from tissue collected from both putative fathers and 10 embryos for each of the 35 replicates that resulted in spawning during Experiment 1. We used primers for two previously developed markers (PPRO 118 and PPRO 171; Bessert & Orti, 2003) to amplify microsatellites in single-primer-pair polymerase chain reactions (PCR) in an Eppendorf Mastercycler gradient thermalcycler under the optimized conditions suggested by Bessert & Orti (2003). Forward primers were labelled with fluorescent dye (HEX, 6FAM), and PCR products from each locus were combined within an individual or clutch for multiplex fragment

analysis. We successfully isolated DNA from both the tissue and embryos for 26 of 35 replicates. Paternity for each replicate was determined by visual inspection of the electropherograms using GeneMapper software (v 3.75; Applied Biosystems). We used DNA fingerprinting analysis (sensu Burke et al., 1989; see also Avise, 2004) because paternity had to be determined for only two individuals and maternity was known for all replicates. Briefly, because we did not genotype the females from each replicate, those bands (=alleles) in the progeny that could not have been inherited from either putative father were identified as maternally derived and excluded from our analysis. We then scored the potential contribution of each male to the observed clutch genotype by counting the number of shared bands at each locus and calculating the probability of paternity using the formula

$$\Pr[\text{Male}_i] = (b_{m1} \times b_{m2})/(b_1 \times b_2),$$

where b_{m1} is the number of bands shared by the embryos and the male at the first locus, b_{m2} the number of bands shared by the embryos and the male at the second locus, b_1 is the total number of paternally derived bands observed in the embryos at locus_1 and b_2 is the total number of paternally derived bands in the embryos at locus_2.

Results

Experiment 1. Sexual selection during initial spawning

Males used in this experiment were 38.9 to 75.15 mm in standard length ($\bar{x} \pm$ SD: 50.72 ± 6.07) and 1.63 to 9.59 g ($\bar{x} = 3.20 \pm 1.30$) in wet mass. Banding pattern expression ranged from 1 (no visible banding) to 4 (dark banding; Unger, 1983) and mean banding pattern expression was moderate among the males ($\bar{x} = 2.52 \pm 0.93$). Dorsal pad development ranged from 1 (beginning expression of the dorsal pad) to 3 (complete development; Unger, 1983); only three individuals expressed a dorsal pad score below two. All males had fully developed tubercles (score = 4; Unger, 1983), but differed in the number of tubercles expressed, ranging from 10 to 39 ($\bar{x} = 22 \pm 5$).

Male–male competition

Body size and secondary sex characters significantly affected the probability that a male would succeed at acquiring a nest site (Whole Model: $F = 6.095$,
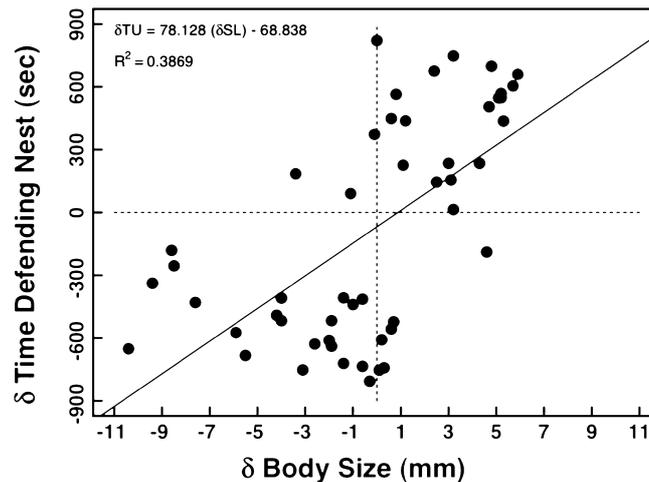
Figure 3. Relationship between the difference in body size between males and the difference in the time each male spent guarding the nest during the male–male competition experiment.

df $= 4$, $p = 0.001$). The best morphological predictor of male success in intrasexual contests was body size ($F = 19.116$, df $= 1$, $p = 0.0001$; Figure 3), with no influence of banding pattern ($F = 1.204$, df $= 1$, $p = 0.28$, $R^2 = 0.000$), dorsal pad development ($F = 0.303$, df $= 1$, $p = 0.59$, $R^2 = 0.007$), or tubercle number ($F = 0.289$, df $= 1$, $p = 0.60$, $R^2 = 0.044$).

Female choice

Female fathead minnows spawned with males that won their intrasexual contest during 57% (20/35) of our trials, which did not differ significantly from the expected frequency of 50% (two-tailed binomial test, $p = 0.69$).

Although female fathead minnows did not preferentially spawn with males that won intrasexual contests, male morphology was associated with reproductive status. Specifically, large male body size (mean difference $= 2.21$, $t = 2.93$, df $= 34$, *$p = 0.0061$; Figure 4A) and a dark banding pattern (mean difference $= 0.579$; df $= 34$; *$p = 0.0016$; Figure 4B) were associated with male reproductive success, whereas dorsal pad development (mean difference $= 0.014$, df $= 34$, $p = 0.82$) and tubercle number (mean difference $= 1.03$, df $= 34$, $p = 0.34$) were not.
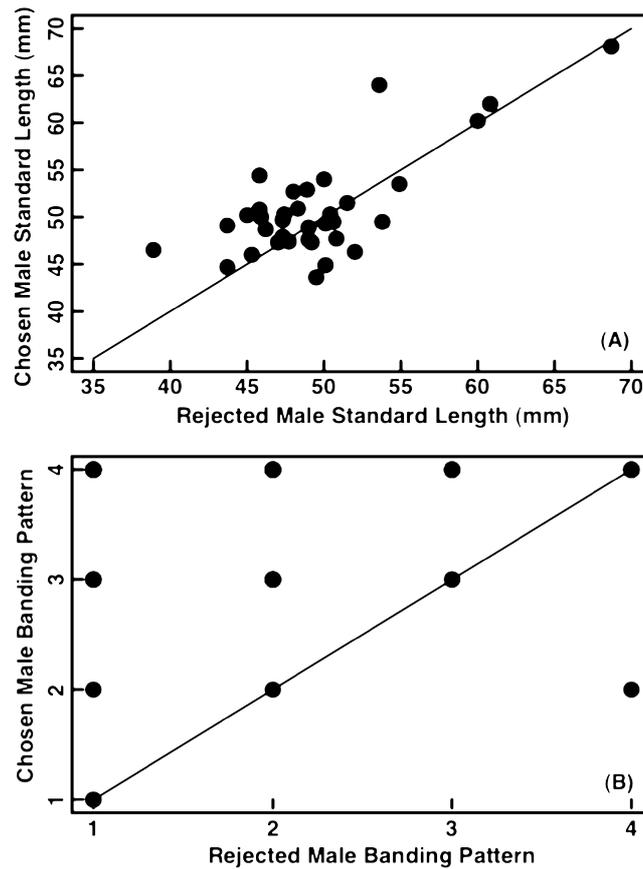
Figure 4. Results of multiple matched-pairs analyses of morphological characters and their association with male mating success. Body size (A, $p = 0.0061$) and banding pattern (B, $p = 0.0016$) were significantly associated with male reproductive success.

Male behaviour and female choice

Males that were chosen by females spent 86% of their time ($\bar{x} = 492 \pm 144$ s) guarding their nests and made an average of 3.9 ± 4.4 approaches toward the female during the female choice stage of our experiment. Rejected males spent 71% of their time ($\bar{x} = 426 \pm 204$ s) guarding their nests and made an average of 1.9 ± 3.1 approaches toward the female. There were no significant differences between chosen and rejected males in nest attentiveness and courtship (multiple logistic regression, $\chi^2 = 3.65$, df $= 2$, $p = 0.16$).

Paternity analysis

We successfully amplified two microsatellite loci (PPRO118 and PPRO171; Bessert & Orti, 2003) for 74.2% (26/35) of the replicates that yielded a clutch of eggs during Experiment 1. Of these, paternity was confirmed for 100% (26/26) of the males observed to be guarding the eggs (Table 1).

Experiment 2. Male reproductive history and female choice

Female fathead minnows chose to spawn with the previously mated male in 45% (9/20) of the choice trials. Hence, female preference for a given male fathead minnow was not predicted by his reproductive history (log likelihood $\chi^2 = 0.20$, df $= 1$, $p = 0.655$). As in Experiment 1, however, female fathead minnows chose to spawn with the larger of the two males in 85% (17/20) of the choice trials. Thus, the result that female fathead minnows prefer relatively large males was independently corroborated with our second experiment (log likelihood $\chi^2 = 10.818$, df $= 1$, $p = 0.001$). The probability of significant female preference for male body size arising in two independent experiments is small (Fisher's Combined Probability Test, $\chi^2 = 26.57$, df $= 4$, $p < 0.0001$; Sokal & Rohlf, 1995).

Discussion

Body size and mating success

Among the traits we measured, body size was the best predictor of male success during both inra- and intersexual phases of mating (Figures 3A and 4A), suggesting complimentary sexual selection on body size. Dominant males, however, did not realize greater mating success (one-tailed binomial test: $p = 0.69$), suggesting a non-complimentary interaction between the two modes of selection. On the one hand, our open aquarium design, although a good proxy for processes in natural populations, raises the possibility that female preference decisions were affected because male–male interactions were not explicitly prevented; e.g., winner-loser effects (sensu Hsu & Wolf, 1999) or interference competition (e.g., Kodric-Brown, 1992; Candolin, 1999; Petersson et al., 1999). On the other hand, our result might reflect contrasting interactions between intra- and intersexual processes of sexual selection.

Table 1. Results for paternity analysis by DNA fingerprinting for 26 replicates for which there were female choice data and we were able to successfully amplify microsatellites from both putative fathers and a sample of the eggs. For the eggs, the numbers indicate the number of paternally derived alleles at each of two microsatellite loci (Loc 1 and Loc 2). For the chosen and rejected male of each female preference replicate, the numbers indicate the number of alleles shared with the eggs at each microsatellite locus, and the probability of paternity (P) is indicated for each male.

Rep	Eggs		Rejected male			Chosen male		
	Loc 1	Loc 2	Loc 1	Loc 2	P	Loc 1	Loc 2	P
1	1	2	0	1		1	2	1.00
2	1	1	0	0		1	1	1.00
3	1	1	0	1		1	2	1.00
4	1	2	0	0		1	2	1.00
5	1	1	0	1		1	1	1.00
6	2	3	1	1	0.17	2	3	1.00
7	2	2	1	0		2	2	1.00
8	1	2	0	0		1	2	1.00
9	3	2	2	0		1	2	0.33
10	1	1	0	0		1	1	1.00
11	1	3	1	1	0.33	1	3	1.00
12	1	1	0	0		1	1	1.00
13	1	2	0	1		1	2	1.00
14	1	2	1	1	0.50	1	2	1.00
15	1	2	1	1	0.50	1	2	1.00
16	1	2	1	0		1	2	1.00
17	2	2	0	0		2	2	1.00
18	1	3	0	2		1	2	0.67
19	1	2	1	0		1	2	1.00
20	2	2	1	0		2	2	1.00
21	1	1	1	0		1	1	1.00
22	1	2	0	0		1	2	1.00
23	1	3	0	1		1	2	0.67
24	1	2	1	1		1	2	1.00
25	1	3	0	1		1	2	0.67
26	1	2	0	0		1	2	1.00

If dominance relations between males remained unchanged over the duration of our experiment, and dominance relations interfered with female preference, then our result should have been complete agreement between dominance status and mating success. This, however, was not the case. More-

over, our behavioural data suggested that, although male–male interactions did occur during the female preference stage of our experiment, they were of relatively low intensity in comparison to the interactions during the intrasexual contest stage, and that dominance relations remained consistent throughout the duration of each replicate (SPH, personal observation). Furthermore, post hoc analysis of data from Experiment 2 using a no choice approach (e.g., Shackleton et al., 2005) with mating treatment as the predictor variable and latency until spawning as the response variable did not support the hypothesis of interference with female preferences from male–male competition: during the preparation stage of Experiment 2, the mean latency until spawning for smaller males was nearly double that of larger males ($\bar{x} = 9.78$ days and $\bar{x} = 5.78$ days for smaller and larger males, respectively). This result suggests a significantly greater propensity for female fathead minnows to spawn with larger males (*t*-test assuming unequal variance: $t = 2.743$, df $= 16$, $p < 0.02$). Further, observations of female behaviour during the female choice portion of our experiment did not suggest any interference by males in female mate choice decisions. Hence, we do not believe that the congruent advantage of large body size was the result of an influence of our open aquarium design. Why, then, did females not select large, dominant males more often? We suggest that the answer reflects the difference in size between the males being compared.

Data from both of our experiments suggested a threshold size difference of ∼5 mm, below which body size was not predictive of either dominance (Figure 5A) or female preference (Figure 5B). When body size differences were less than approximately 2 mm, however, subordinate males may have had greater mating success than did dominant males (Figure 5C). Hence, it appears that when body size differences are large (i.e., greater than 5-10%), larger males are favoured, perhaps because they are both better at attaining and defending territories (e.g., Danylchuk & Tonn, 2001) and providing direct fitness benefits to females via greater egg survival (sensu Bisazza et al., 1989). Below a difference of approximately 2 mm, however, it seems that females might preferentially choose subordinate males. Our results suggest that intra- and intersexual processes of sexual selection are complimentary when differences between males are above certain thresholds, but also that there is opportunity for different levels of interaction when males are similar with respect to size. It is this potential for opposing intra- and intersexual selection processes that might prevent the fixation of alleles, especially if the strength of the two forces is spatially and temporally variable.
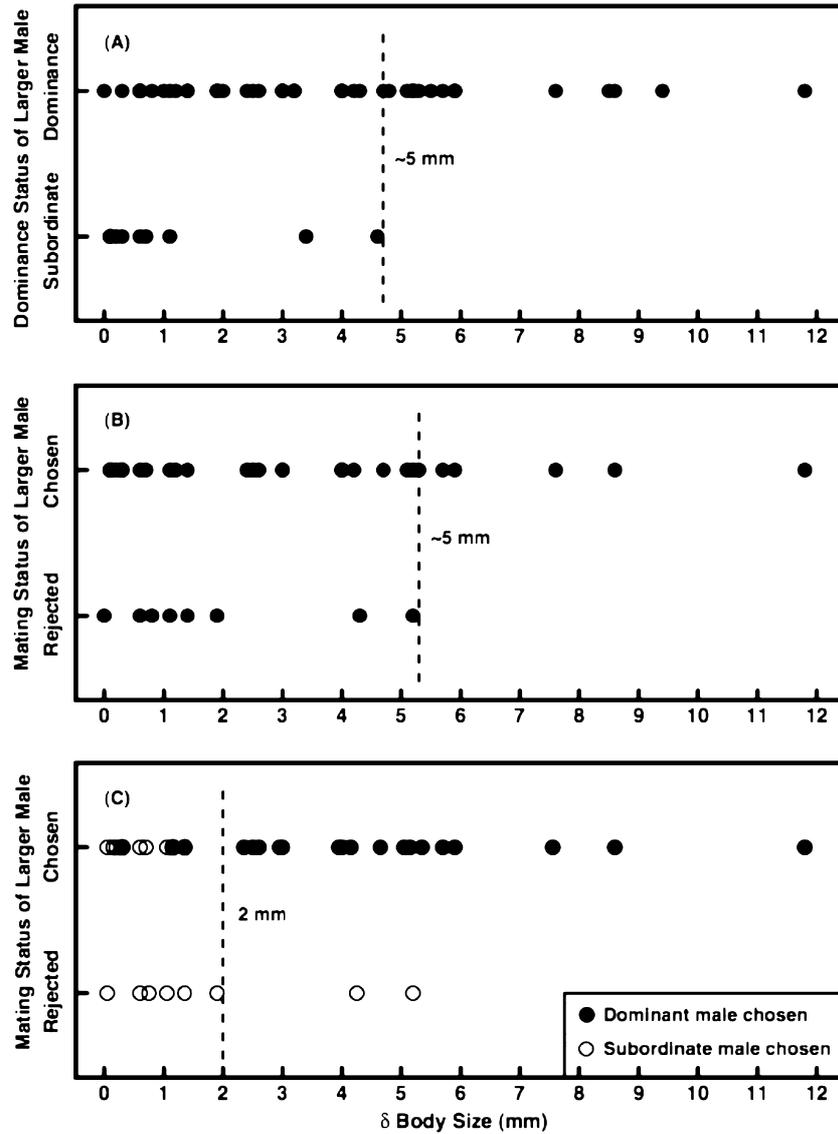
Figure 5. Graphical comparisons of male success on body size difference during both intra and intersexual stages of our experiments. Overall, large male body size was favoured during intrasexual contests (A), and intersexual mate choice (B), but was absolutely predictive only over a threshold size of approximately 5 mm. Below 2 mm females apparently preferred to mate with subordinate males (C) suggesting that the interaction between processes of sexual selection shift with respect to the size structure of the mating population.

Secondary sex characters and mating success

Secondary sex characters had no detectable influence on the outcome of intrasexual contests for male fathead minnows. Banding pattern intensity, however, was significantly associated with male reproductive success (Figure 4B), suggesting that females prefer dark banding patterns and large body size. Unger (1983) speculated that the banding pattern of male fathead minnows enhances the robustness of their appearance, and, thus, discourages other males from attacking and attempting to usurp nests. In our experiments there was no association between banding pattern and success during male–male contests, but two factors complicate the interpretation of this result.

First, during the intrasexual selection stage of Experiment 1, males were scored for banding pattern expression before male–male competition took place. A post hoc examination of banding pattern expression following competition over a nest substrate revealed that dominant males were slightly, but not significantly, darker than their subordinates, and the difference did not persist through the beginning of the female choice stage of Experiment 1 (Hudman, unpublished data). Second, the banding pattern scores used in the analysis of female preference were assigned after the female had deposited eggs. Thus, we cannot eliminate Unger's hypothesis because each reproductively successful male may have increased his banding pattern expression *after* he spawned to prevent attacks from the other male. In concert, however, our data do suggest that males increase their banding pattern expression in response to competitive interactions with other males, and that they may further increase their expression when they are reproductively successful, possibly as Unger (1983) suggested, to discourage attacks from other, unsuccessful males.

Tubercles are used during intense aggressive interactions between males (McMillan & Smith, 1974; Vives, 1988; Hudman, personal observation). Nevertheless, there was little variation among males in their degree of tubercle development, and tubercle number was not associated with success during intrasexual contests or spawning. Thus, the function of nuptial tubercles during fathead minnow reproduction remains unclear. Nuptial tubercles are present in several other species within Cyprinidae, but they are not necessarily expressed in the same pattern or, perhaps, serve the same function as in fathead minnows (e.g., central stoneroller, *Campostoma anomalum*, and horneyhead chub, *Nocomis biguttatus*, Boschung et al., 1983). Because nuptial

tubercles are apparently broadly distributed in the Cyprinidae, it is possible that this trait is phylogenetically constrained and differentially expressed depending on the breeding biology of the species, rather than a secondary sex character that has evolved by sexual selection.

Behaviour and mating success

We found no association between male behaviour toward females and reproductive success. Cole & Smith (1987) suggested that male fathead minnows demonstrated conspicuous female-directed courtship behaviours characterized by males approaching females and leading them to their nests. Pyron & Beitinger (1989), however, found no evidence that male fathead minnows use courtship to attract females. Because all of the behaviours described as courtship in Cole & Smith (1987) would necessarily be preceded by the male approaching the female, we quantified only the frequency of approaches during our behavioural observations. Although reproductively successful males were more attentive to their nests and approached the female more often, there was no association between male behaviour and reproductive success. Thus our behavioural data agree with those of Pyron & Beitinger (1989), who suggested that male fathead minnows do not court females. We do not mean to imply that behaviour is unimportant to male reproductive success. Rather, we suggest that other behaviours (e.g., paternal care behaviours) or general activity level might be more important in attracting the attention of female fathead minnows.

Conclusion

The association between large body size and mating success has been documented in a broad array of animal taxa including birds (Hagelin, 2002), reptiles (Lopez et al., 2002), fishes (Rosenthal & Evans, 1998; Aspbury & Basolo, 2002; Basolo, 2004), insects (Choe & Crespi, 1997) and spiders (Kotiaho et al., 1997), and is common in taxa with resource defence mating systems (reviewed in Andersson, 1994; see also Bisazza & Marconato, 1988; Reichard et al., 2005). Historically, it has been assumed that size is a proxy for dominance status, thus female preference for size is a preference for dominant males (Shackleton et al., 2005). This assumption, however, has been challenged (Qvarnstrom & Forsgren, 1998; Wong & Candolin, 2005),

and a growing number of studies in an array of taxa have lead to a better understanding of the interactions between and relative contributions of intra- and intersexual mechanisms of sexual selection (e.g., Moore & Moore, 1999; Moore et al., 2001; Lopez et al., 2002; Candolin, 2004; Wong, 2004; Shackleton et al., 2005; Borg et al., 2006). Our results from fathead minnow show that large male body size is favoured during male–male competition for nests and is preferred by females choosing mates when size differences between males were greater than 5 mm. When males differed by 2 mm, however, females preferred subordinate males. Female fathead minnows, therefore, may adjust there priorities for choosing mates based on the size structure of the males defending territories, thus maintaining genetic variation for choice criteria.

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