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MALE DOMINANCE, FEMALE MATE CHOICE, AND INTERSEXUAL CONFLICT IN THE ROSE BITTERLING (*RHODEUS OCELLATUS*)

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An intersexual conflict arises when males and females differ in their reproductive interests. Although experimental studies have shown that females often mate with dominant males, it may not always be in the interest of a female to do so. Here we investigated the impact of male dominance on female mate choice and offspring growth and survival in the rose bitterling (*Rhodeus ocellatus*), a freshwater fish with a resource-based mating system. Three experimental mating trials were conducted using males of known dominance rank, but with different levels of constraint on male behavior. Thus, females were able to choose among; (1) males that were isolated from each other; (2) males that could see and smell each other, but could not directly interact; (3) males that could interact fully. Using a combination of behavioral observation and parentage analyses it was shown that female preferences did not correspond with male dominance and that male aggression and dominance constrained female mate choice, resulting in a potential intersexual conflict. The survival of offspring to independence was significantly correlated with female mate preferences, but not with male dominance. A lack of strong congruence in female preference for males suggested a role for parental haplotype compatibility in mate choice.

KEY WORDS: **Female mating preference, good genes, mate choice, MHC, olfactory cues, *Rhodeus ocellatus*.**

Sexual selection arises through intrasexual variance in reproductive success, with variance usually higher in males due to their greater potential reproductive rates, a result of differences in gamete allocation between the sexes. Two main mechanisms of sexual selection are recognized. Intrasexual selection typically involves male–male competition; males actively compete for access to females or resources that are necessary to attract females. Intrasexual selection gives rise to selection for male fighting ability, often including weapons and large body size. Intersexual selection involves some aspect of mate choice, usually by females, with preferences based on direct or indirect (or both) benefits and giving rise to selection for elaborate signals and displays by males and preferences for these traits by females (Darwin 1871; Fisher 1930; Williams 1966; Andersson 1994).

The relative roles of intra- and intersexual selection and their interaction is a key question in sexual selection theory (Kokko 2005). Males that succeed in contests with other males may be attractive to females because they provide direct or indirect benefits of mate choice or both, and male contests may better resolve information that females use in mating decisions (Berglund et al. 1996; Wong and Candolin 2005). Alternatively, dominant males, and the traits associated with male dominance, may not be preferred by females (Qvarnström and Forsgren 1998). Instead females may be constrained in mate choice through male intrasexual selection, and risk a reduction in reproductive fitness when mating with dominant but nonpreferred males, particularly if mate choice is linked to nonadditive genetic benefits or direct costs to female fitness arising through male coercion (Jacob et al. 2007; Reichard et al. 2007a).

Here intra- and intersexual components of sexual selection were experimentally separated in the rose bitterling (*Rhodeus ocellatus*), a small cyprinid fish with a resource-based mating system. To do this, intrasexual competition was allowed to vary at three levels (none, moderate, and high), using males of known dominance rank. Female and male effects on reproductive success were contrasted and matched to male morphological and behavioral traits. In addition, in vitro fertilizations were used to compare the fitness benefits of preferred and nonpreferred mates to females in terms of embryo growth and survival. Two predictions were tested: (1) Whether dominant males (and traits associated with dominance) would be preferred by females, irrespective of the degree to which intrasexual competition was constrained, or whether there was a mismatch between male dominance and female mate preference. (2) Whether female mate preference conferred a fitness benefit to females through increased offspring survival or quality.

In bitterling (*Rhodeus* spp., Acheilognathinae), both intra- and intersexual selection can play a role in the mating system (Kanoh 2000; Candolin and Reynolds 2001; Smith et al. 2002, 2003; Reichard et al. 2005). During the breeding season, males develop red carotenoid-based nuptial coloration, most notably in the iris and on the fins, and compete for territories around living unionid mussels that females use for oviposition. The bitterling mating system is promiscuous; both males and females spawn repeatedly, with multiple partners. Males court females and attempt to lead them to mussels in their territories. Female oviposition decisions are based on both male and mussel quality (Smith et al. 2000b; Candolin and Reynolds 2001; Kitamura 2006; Casalini 2007). Females deposit one to five (typically three) eggs in the mussel gill chamber. Females spawn in several bouts lasting one or two days and consisting of approximately 5–10 independent spawnings each day (Nagata 1985; Smith et al. 2004). Territorial males release sperm over the inhalant siphon of the mussel so that sperm drawn into the gills fertilizes the eggs. Sneaking behavior, in which a rival male (an adjacent territory holder or a male that does not possess a territory) releases his sperm into a rival's mussel, is common in bitterling (Kanoh 1996, 2000; Smith et al. 2002, 2003; Reichard et al. 2004a,b). Male mating behavior is largely opportunistic and there is no evidence of a morphological or genetic distinction between territorial and sneaking males (Kanoh 2000). Females appear able to undermine male dominance, to some extent at least, by soliciting sneakers, delaying spawning, and performing a spawning action but without depositing eggs (Smith and Reichard 2005; Smith et al. 2007). For further details on bitterling reproductive biology see Smith et al. (2004).

Materials and Methods

EXPERIMENT 1: ISOLATED MALES

All experiments were performed in the aquarium facility of the Department of Biology at the University of Leicester during 2007. Fish for experimental work were wild-caught *R. ocellatus* from the River Yangtze Basin, China. Experimental fish were raised in captivity and were between 30–34 months of age during experiments. Prior to experiments fish were kept in a single aquarium measuring 120 (length) × 40 (width) × 45 (depth) cm and fed a mixture of frozen bloodworm (*Tubifex* spp.) and commercial dried fish flake food twice each day and live zooplankton three times each week. Stock and experimental aquaria were on a recirculating system with water temperature in all aquaria at 23°C. Each aquarium contained a 20 mm layer of sand substrate and fish were exposed to a 16 h: 8 h light/dark regime. *Rhodeus ocellatus* readily spawn in *Unio pictorum* (Casalini 2007), and this species of mussel was used in all experiments. Fish were not reused between different experiments. Experimental mussels were collected from the River Cam and stored in a 100-L tank on the roof of the Department of Biology and fed live phytoplankton daily.

Before all experimental trials, groups of experimental males were ranked for dominance. To do this, four males were haphazardly selected from the stock aquarium and placed in an experimental aquarium measuring 60 (length) × 40 (width) × 40 (depth) cm with a female in spawning condition (with a fully extended ovipositor) and a mussel in a sand-filled flower pot covered with a perforated transparent plastic cup to allow visual and olfactory inspection of the mussel but not spawning. Two artificial plants were placed in the aquarium as refuges. The four males remained together for 1 h to enable dominance to establish; the dominant male aggressively excluded other males from approaching the mussel. Once dominance was established the dominant male was removed. The same procedure was repeated to identify the second, third, and fourth-ranked males. Dominance in bitterling, once established, is highly stable (Reichard et al. 2005) and can last for at least six weeks under natural conditions (Smith et al. 2000b).

After completion of ranking, males were placed singly in a small aquarium measuring 25 (length) × 40 (width) × 30 (depth) cm with a single *U. pictorum*. The following morning, four females in spawning condition were randomly assigned to each male. After 1 h, the mussels were removed and gently opened, nondestructively, with a mussel-opening device (Kitamura 2005), and the number of eggs that had been spawned were counted. Each female was moved to a different aquarium with a different ranked male following a random predetermined pattern. This process was repeated three times so that, at the end of the trial, each female had been paired once with each of the four males following the design of Spence and Smith (2006). To exclude any effect on oviposition of a preference for mussels when females were moved, they were accompanied by the same mussel. The number of eggs a mussel can host in its gills is higher than a single female can spawn in a day, even with repeated spawning events (Smith et al. 2004), and the presence of a limited number of eggs in the gill cavity of mussels was not predicted to interfere with spawning decisions (Smith et al. 2001; Kitamura 2005, 2007; Reichard et al. 2007b). At the end of each trial the size (measured from the tip of the snout to the origin of the tail fin) of females and males was measured to the nearest 1 mm with electronic calipers. In addition, the left and right eye and tail fin spot of every male was photographed using a Canon EOS 300D digital camera with a 60 mm macro lens under standard light conditions.

After completion of trials, fish were housed in a separate stock aquarium and were not used again. Mussels containing eggs were placed in tanks with phytoplankton to allow the embryos to complete development. Mussels were used only once in the experiment. A total of seven replicate trials were completed using 28 males, females, and mussels.

The area of red color in the iris of males was measured using a protocol modified from that of Barber et al. (2000). Images were analyzed using Photoshop 2.0 (Adobe Systems Inc., San Jose, CA). Using the magic wand facility, a single pixel was chosen from the area of red in the iris and successive portions of the image were selected based on color similarities until all the pixels within the red-colored area had been captured. The total area of the iris, excluding the pupil was similarly estimated and the red area was expressed as a proportion of the total. There was no significant difference in the red area of the iris between the right and left eye of males (paired t-test: $t_{28} = 1.42$, $P = 0.170$). Consequently, data for the mean of the left and right eyes were used for analysis. MC, who was blind to male dominance rank, visually scored the extent and brightness of red color of the tail fin spot on a scale from 0 to 5 using criteria in Table 1. In addition, to address concerns of subjectivity and reliability of this measure, two of us (CS and MA) independently scored a random subsample of 20 images using the same ordinal scale in Table 1. No significant difference in color ranking was detected among the three experimenters (analysis of variance [ANOVA] $F_{2,57} = 0.18$, $P = 0.836$). As a test of repeatability of the two most divergent scores (those of CS and MC), the standard deviation of differences between repeated measurements was obtained for each experimenter and the standard deviation of the differences between means. The corrected standard deviation was estimated to be 0.778 using the method of Bland and Altman (1999). If 95% of differences in color ranking between experimenters lie within 1.96 standard deviations of this estimate then differences between the measurements of each experimenter can be considered interchangable (Bland and Altman 1999). In the present case all differences in color scores lay within this margin, suggesting high repeatability. Color variation in laboratory-reared fish was variable and comparable to that detected in color measurements of wild bitterling (Smith et al. 2002).

EXPERIMENT 2: CONSTRAINED MALES

Before each trial, groups of four haphazardly selected males were ranked for dominance in the way described for experiment 1. After ranking, males were placed in an experimental aquarium measuring 60 (length) × 40 (width) × 40 (depth) cm divided into four compartments, each measuring 30 (length) × 20 (width) cm, separated with walls of thin wire mesh measuring 5 × 5 mm. Males were placed alone in compartments with a size-matched mussel in a sand-filled pot and an artificial plant. Male *R. ocellatus* are larger and deeper bodied than females and the mesh walls prevented males passing into adjacent compartments but enabled smaller females to pass through freely. Males could see and smell individuals in neighboring compartments.

The following morning, after covering all mussels with a transparent perforated plastic cup, a female in spawning condition was placed in a randomly selected compartment of the experimental aquarium. After 2 h the mussels were uncovered and fish behavior was scored for 20 min using a palm computer with the FIT-system behavior recording software (Held and Manser 2005). After a further 2 h the mussels were covered again for a further 2 h and the procedure was repeated. The behaviors scored were: (1) frequency of female inspection of mussels (the fish positions its snout close to the exhalant siphon of the mussel), and (2) rate of

Table 1. Ordinal scale of the extent of red coloration of male caudal fin spot in *Rhodeus ocellatus*.

Ordinal rank	Descriptor
5	An area of brilliant red extending from the body to the hind edge of the tail
4	An area of brilliant red extending from the body for $^3/_4$ of the length of the tail fin
3	An area of brilliant red extending from the body to $^1/_4$ – $^1/_2$ of the length of the tail fin
2	An area of pale red extending from the body to $^3/_4$ of the length of the tail fin
1	An area of pale red extending from the body to $^1/_4$ – $^1/_2$ of the length of the tail fin
0	Only pale traces of red or an absence of color on the tail fin

male courtship (the male swims toward a female then approaches a mussel while "standard length (quivering") (Smith et al. 2004). At the end of each day female SL) was measured and the female was placed in a stock aquarium and was not used again in the experiment. The number of eggs laid by the female was quantified using a mussel-opening device.

The same procedure was repeated on four consecutive days with a different female each day. All males and mussels were randomly assigned to a different compartment independently on each day of a trial. At completion of a trial, male body length was measured with electronic calipers and the left and right eye and tail fin spot of every male were photographed. After measurements were completed, fish were returned to a stock aquarium and were not used again. Experimental mussels were measured (maximum shell length) to the nearest 1 mm and moved to large tanks with phytoplankton to allow the embryos to complete development and were not used again in the experiment. The area of red color in the iris and extent and brightness of red color of the tail fin spot were quantified using the same procedure for experiment 1. A total of six replicates were completed over a six-week period using 24 males, females, and mussels.

EXPERIMENT 3: UNCONSTRAINED MALES

Groups of four haphazardly selected males were ranked for dominance in the way described for experiment 1. After ranking, males were placed in an experimental aquarium measuring 60 × 40 × 40 cm with a single *U. pictorum* placed in a sand-filled pot. Characteristic features of males were recorded to enable them to be individually identified (Kanoh 1996; Smith et al. 2002, 2003). The following morning, after covering the mussel with a transparent perforated plastic cup, a female in spawning condition was released in the aquarium. After 1 h the mussel was uncovered and the fish was videoed for 20 min. After 2 h the mussel was covered again for 1 h and the procedure was repeated, with the fish allowed to spawn for a further 2 h. Behavior was later scored from video recordings for each fish separately, and included: (1) rate of female inspection of the mussel; (2) frequency of spawning; (3) rate of male aggressive defense of the mussel; (4) rate of male courtship; (5) frequency of ejaculations 2 min before and after a spawning event; ejaculations during this period have the highest probability of resulting in successful fertilizations (Kanoh 1996; Reichard et al. 2005).

After completion of a trial the mussel was measured and checked for eggs. If eggs were present the mussel was dissected and the eggs were incubated in a Petri dish for five days. After five days embryos were fixed in ethanol for parentage analysis. Female SL was measured to the nearest 1 mm and a small portion of the tail fin was removed and fixed in ethanol. The experimental procedure was followed with the same group of males for four days with a different female used on each day. After this period, males were measured and photographed in the way described for experiment 1 and a small portion of the tail fin was removed from each male and fixed in ethanol. Fish were returned to a stock aquarium and were not used again in the experiment. The area of red in the iris and extent and brightness of red on the tail fin spot were quantified using the same procedure for experiment 1. A total of eight replicates were completed using 32 males, females, and mussels.

For parentage analysis DNA was extracted from ethanol-preserved tissue using the methods of Reichard et al. (2008). A subset of samples from adult fish was initially genotyped for 12 variable microsatellite loci developed for the closely related *Rhodeus amarus*; *Rser01–06*, *Rser08–Rser12* (Dawson et al. 2003), and *Rser13* (Reichard et al. 2008). Based on their informative value and compatibility, eight loci were combined in two multiplex PCR reactions (Set I—*Rser03*, *04*, *09*, *13* and Set II—*Rser01, 02, 05, 12*), with a mean of 11 (range: 5–17) alleles per locus. For details see Reichard et al. (2008). The length of the DNA fragments was analyzed using GeneMapper® software. Observed heterozygosities enabled parental assignment by the exclusion of incompatible paternal and maternal genotypes using Cervus 3.0 (Kalinowski et al. 2007).

A total of 266 embryos were collected for parentage analysis. In one replicate only three eggs were recovered and data for this replicate were excluded from the subsequent analysis. All other embryos were analyzed, with a mean of 36 (range: 10–67) embryos per replicate. Paternity was assigned with 95% confidence in all offspring except two from the same replicate. In this case paternity could not be assigned with confidence and those embryos were excluded from the subsequent analysis.

EXPERIMENT 4: CONSEQUENCES OF MATE CHOICE

Groups of four haphazardly selected males were ranked for dominance in the way described for experiment 1. The same experimental procedure for experiment 2 was repeated, but with the following modification: each replicate comprised four males placed in individual chambers but only a single female was allowed to choose among males, with the number of eggs laid by females used as a measure of female mate choice. After completion of the mate choice trial the female was isolated until she ovulated another batch of eggs, an interval of 5–7 days (Smith et al. 2004). Eggs were stripped from the female by gently squeezing her abdomen and divided into four batches of equivalent size. The eggs were fertilized within 5 min. using sperm stripped from the same four males used in the mate choice trial. Stripping sperm from males yields an excess of sperm and all eggs were successfully fertilized, developed a perivitelline space, and began the initial development. The eggs were incubated in a 70-mm-diameter Petri dish in freshwater and a record was kept of their survival over a period of four weeks, after which time the larvae achieve independence. After four weeks larvae were photographed under standard light conditions next to a scale bar and their length was estimated to the nearest 0.1 mm from digital images.

DATA ANALYSIS

All data were tested for normality using a Kolmogorov–Smirnov test and for equality of variance using Bartlett's test. Data that did not meet assumptions of normality and homoscedasticity were transformed. In experiment 1, to test for male, female, and order of spawning effects on number of eggs laid a nested ANOVA with experimental males, females, and the order of spawning nested within replicates and trial number as a repeated measure was used (see Spence and Smith 2006). In experiments 2 and 3, to test for male and female effects on numbers of eggs laid and parentage, respectively a nested ANOVA with experimental males and females nested within replicates was used. A significant female effect in this analysis indicated a bias in the number of eggs laid by females toward specific males, whereas a significant male and female effect showed congruence among females in the spawning bias. A male effect alone indicated a significant bias in the number of eggs laid or fertilized by males, but not one that arose through a consistent bias among females. A comparison was made among experiments 1–3 in female clutch size to test for an effect of female allocation of eggs to degree of male constraint. Mean clutch size was compared using analysis of covariance (ANCOVA) with female size as covariate. In other analyses, one-way ANOVAs (with Tukey's test for pairwise posthoc comparisons) and Pearson's correlations were used to test differences and associations between morphological and behavioral data, dominance, and reproductive success. If data transformation did not improve the data distribution to meet assumptions of parametric tests, nonparametric equivalents (Kruskal-Wallis test, Spearman correlation) were used.

Results

EXPERIMENT 1: ISOLATED MALES

Body size but not red coloration was the predictor of male dominance status. There was a difference in mean body size among male ranks (one-way ANOVA: $F_{3,24} = 27.32$, $P < 0.001$; Fig. 1), although no relationship was found between male dominance rank and the extent of red area of the iris (one-way ANOVA, $F_{3,24} =$

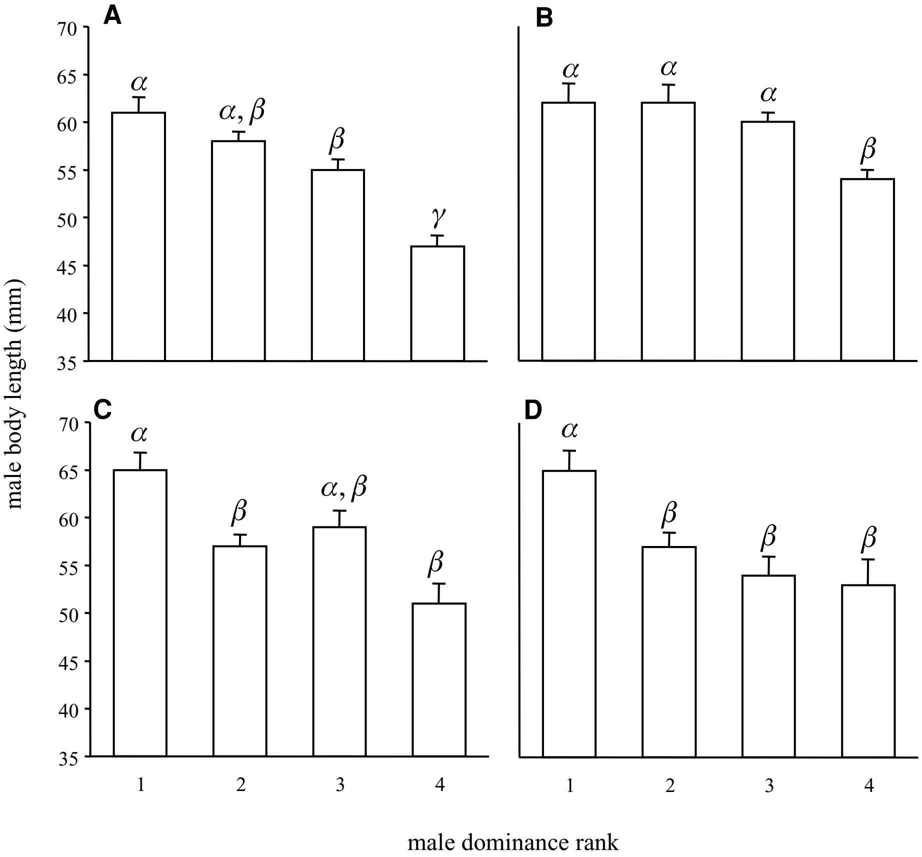

Figure 1. Mean (±SE) body length of males in relation to dominance rank in (A) experiment 1, (B) experiment 2, (C) experiment 3, (D) experiment 4. Lower case Greek letters above error bars denote significantly different groups as determined by posthoc pairwise comparisons; values with the same letter did not differ significantly.

1.22, $P = 0.326$) or the extent of tail spot redness (Kruskal–Wallis test: $H = 0.18$, df = 3, $P = 0.910$).

The mean ± SE number of eggs laid by females over one day of matings was 33 ± 9.4 and varied from 12 to 79. In individual 1-h spawning bouts with individual males, females laid between 0 and 27 eggs with a mean ± SE of 8 ± 1.7 eggs. Females showed a preference for spawning with particular males by laying different number of eggs among males (female effect in nested ANOVA: $F_{21,42} = 3.16$, $P = 0.001$) and were consistent in their preferences for males within replicates, with preferences for specific males (male effect in nested ANOVA: $F_{21,42} = 1.83$, $P = 0.047$). However, these preferences were not related to male dominance rank (one-way ANOVA, $F_{3,24} = 0.55$, $P = 0.650$) or the extent of red coloration (red area of the iris: one-way ANOVA, $F_{3,24} = 1.22$, $P = 0.326$; extent of tail spot redness: Kruskal–Wallis test: $H = 0.18$, df = 3, $P = 0.910$). There was no correlation between male size and number of eggs spawned with a male (Pearson's correlation: $r_{22} = 0.02$, $P = 0.919$).

EXPERIMENT 2: CONSTRAINED MALES

Body size (one-way ANOVA: $F_{3,20} = 7.28$, $P = 0.002$; Fig. 1) and extent of red area of the iris ($F_{3,20} = 6.02$, $P = 0.004$) were positively related to male dominance rank. There was no difference in the extent of tail spot redness among male ranks (Kruskal–Wallis test: $H = 2.01$, df = 3, $P = 0.571$). Females showed preferences for specific males (female effect in nested ANOVA, $\log_{10} + 1$ transformation: $F_{18,36} = 1.97$, $P = 0.041$), but female preferences were not congruent (male effect in nested ANOVA, $\log_{10} + 1$ transformation: $F_{18,36} = 1.30$, $P = 0.248$). Male dominance had no effect on female spawning decision; there was no difference between the numbers of eggs spawned by females among male ranks (one-way ANOVA, square-root transformation: $F_{3,20} = 0.05$, $P = 0.985$).

For behavioral data there was no difference in female mussel inspection rate among male ranks (one-way ANOVA, $\log_{10} + 1$ transformation: $F_{3,92} = 0.14$, $P = 0.935$), and no relationship between mean courtship rate and male rank (one-way ANOVA: $F_{3,20} = 0.26$, $P = 0.852$). However, there was a correlation between the number of eggs laid by females and courtship rate (Pearson's correlation, square-root transformation: $r_{22} = 0.438$, $P = 0.032$; Fig. 2). There was no correlation between courtship rate and the mean extent of red area of the iris of males (Pearson's correlation: $r_{22} = 0.116$, $P = 0.558$). Although courtship rate correlated with redness of the tail fin spot (Spearman's correlation: $r_{22} = 0.411$, $P = 0.046$) there was no correlation between the number of eggs laid by females and extent of redness in the tail fin spot (Spearman's correlation: $r_{22} = 0.203$, $P = 0.341$).

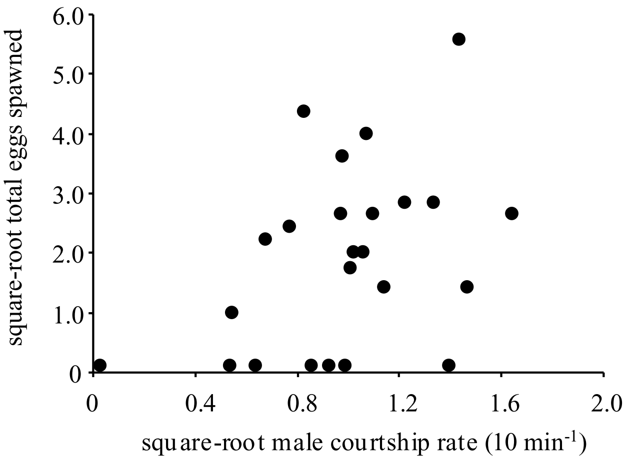

Figure 2. Correlation between the square-root male courtship rate and number of eggs spawned by females in experiment 2.

EXPERIMENT 3: UNCONSTRAINED MALES

Body size predicted male dominance status (one-way ANOVA: $F_{3,24} = 12.01$, $P < 0.001$; Fig. 1), but there was no effect of red coloration (red area of the iris: one-way ANOVA: $F_{3,24} = 0.93$, $P = 0.443$; tail spot redness: Kruskal–Wallis test: $H = 5.45$, df = 3, $P = 0.142$). The number of eggs fertilized varied among males (male effect in nested ANOVA, square-root transformation: $F_{6,31} = 7.07$, $P < 0.001$); dominant males fathered more eggs than lower ranked males (one-way ANOVA, square-root transformation: $F_{3,24} = 4.47$, $P = 0.013$, Fig. 3). There was no female effect on the number of embryos fertilized among males (nested

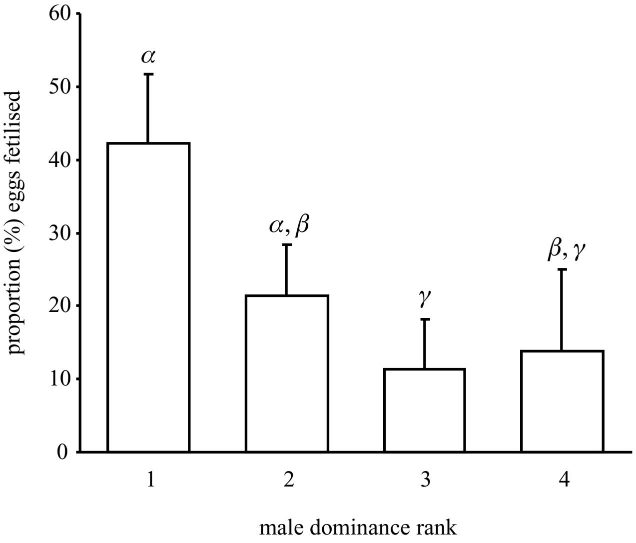

Figure 3. Mean (±SE) male reproductive success among dominance ranks in experiment 3. Lower case Greek letters above error bars denote significantly different groups as determined by posthoc pairwise comparisons; values with the same letter did not differ significantly.

ANOVA, square-root transformation: $F_{6,31} = 1.32$, $P = 0.269$), and no difference in the number of embryos recovered among replicates (nested ANOVA, square-root transformation: $F_{6,31} = 1.89$, $P = 0.113$).

There was a difference in the courtship rate among male ranks (Kruskal–Wallis test: $H = 17.28$, df = 3, $P = 0.001$) with the dominant males courting females at a higher rate than other males. There was a correlation between reproductive success and courtship rate (Pearson's correlation, square root transformation: $r_{26} = 0.466$, $P = 0.012$). There was also a difference in total ejaculation rate among male ranks (one-way ANOVA: $F_{3,24} = 6.87$, $P = 0.002$) with the dominant males ejaculating at the highest rate (Tukey's test: $P < 0.05$).

Mean female clutch size did not vary among experiments 1–3 after adjusting for female size as a covariate (ANCOVA $F_{2,80} = 0.19$, $P = 0.828$; female size was not a significant covariate ANCOVA $F_{1,80} = 2.43$, $P = 0.123$).

EXPERIMENT 4: CONSEQUENCES OF MATE CHOICE

Male body size (one-way ANOVA: $F_{3,28} = 5.84$, $P = 0.003$; Fig. 1) and extent of tail spot redness (Kruskal–Wallis test: $H = 8.81$, df = 3, $P = 0.032$) were positively related to male dominance rank. There was no difference among male ranks in the extent of red area of the iris ($F_{3,28} = 0.83$, $P = 0.491$). There was no difference between the number of eggs spawned by females among male ranks (one-way ANOVA, square-root transformation: $F_{3,28} = 0.84$, $P = 0.482$), and no correlation between female mate preference and male color (Pearson's correlation: red area of the iris $r_{30} = 0.042$, $P = 0.820$; Spearman's correlation: tail spot redness $r_{30} = 0.066$, $P = 0.722$). However, there was a correlation between male courtship frequency and the number of eggs spawned by females with a male (Pearson's correlation, square-root transformation: $r_{30} = 0.537$, $P = 0.002$).

The survival of eggs was positively correlated with female preference for males, one (Pearson's correlation, rank transformation: $r_{30} = 0.455$, $P = 0.009$), two ($r_{30} = 0.379$, $P = 0.032$), three ($r_{30} = 0.378$, $P = 0.033$) and four weeks after fertilization ($r_{30} = 0.449$, $P = 0.010$; Fig. 4). There was no correlation between female mate preference and embryo size after four weeks (Pearson's correlation, rank transformation: $r_{30} = -0.195$, $P = 0.487$). There was no difference in offspring survival among male dominance ranks after four weeks (one-way ANOVA: $F_{3,28} = 0.84$, $P = 0.482$) and no correlation with male color (Pearson's correlation: red area of the iris $r_{30} = -0.023$, $P = 0.902$; Spearman's correlation: tail spot redness $r_{30} = -0.018$, $P = 0.922$).

Discussion

The aim of this study was to investigate the interaction between male intrasexual competition with intersexual mate choice in the rose bitterling, a fish with a resource-based mating system. It was shown that when females were able to select males for mating sequentially (experiment 1) or simultaneously (experiment 2) without interference through male–male competition, they demonstrated preferences for specific males, measured in terms of the number of eggs they deposited. In the case of sequentially presented males (experiment 1) female mating preferences were congruent; females corresponded within replicates in their choice of males. However, female mate choice did not correspond with male dominance rank, size, or coloration (extent of red in the iris or on the tail fin). The only male trait that correlated with female choice was the vigor of male courtship behavior (experiments 2 and 4). When males were able to compete their reproductive success was linked to dominance; first- and second-ranked males fathered the most offspring, although the third- and fourth-ranked males were also successful in fertilizing a proportion of eggs through sneaking (experiment 3). In contrast, the distribution of fertilizations among males did not vary significantly among females; females were unable to exercise mate choice. Female preference for males correlated positively with embryo survival to independence (experiment 4), but there was no effect of male dominance on offspring survival. No effect of male dominance, color, or female preference on offspring size was detected, although density effects on embryo growth rates cannot be excluded from the experimental design used.

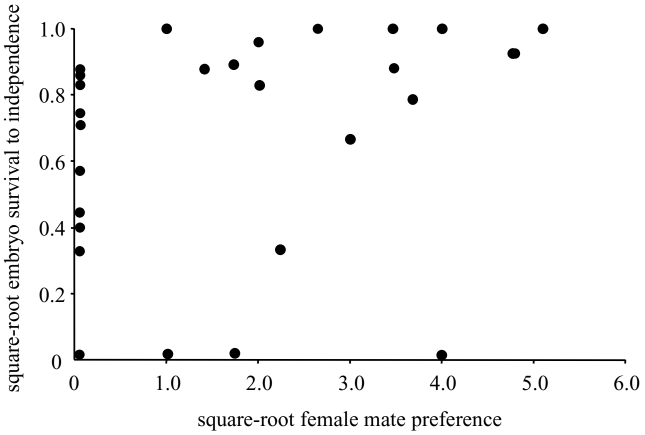

Figure 4. Correlation between square-root female mate preference (number of eggs spawned with males in mate choice trials) and egg survival to independence in experiment 4.

Ostensibly these results suggest a conflict between intra- and intersexual selection, with female mate choice overridden by male dominance. A conflict between these two forms of sexual selection has been argued previously (Moore and Moore 1999; Arnqvist and Lowe 2005). Reichard et al. (2005) used the related European bitterling (*R. amarus*) to show that traits selected in intrasexual selection did not correspond with those selected through intersexual selection. Spence and Smith (2006) demonstrated that male dominance did not match female mate choice in the zebrafish (*Danio rerio*), and comparable results have been obtained for water striders (*Aquaris remigis*) (Sih et al. 2002) and brown trout (*Salmo trutta*) (Petersson et al. 1999). However, in some instances where females are free to choose mates they do select dominant males (reviewed by Wong and Candolin 2005). Consequently, there appears a disparity between mating systems in which female resistance to male dominance has evolved (through so-called "chase-away" selection) and the conventional view of sexual selection that arises through direct, but especially indirect, benefits to mate choice. However, although a distinction between these forms of sexual selection has been drawn, the functional difference between them has been questioned (Kokko 2005).

Despite constraints placed on them by male dominance, females may still find opportunities to mate with subordinate males, for example through extra-pair copulations (Mulder et al. 1994; Forstmeier et al. 2002), avoidance of dominant males (Kangas and Lindström 2001; Bro-Jørgensen 2003), or by increasing mating opportunities for subordinates by delaying oviposition (Smith and Reichard 2005; Smith et al. 2007). Females may also directly demonstrate preferences for subordinates. In coho salmon, *Oncorhynchus kisutch*, females performed more digging behavior (a measure of mate choice) when accompanied by sneaker males and oviposited for longer (and therefore, perhaps, laid more eggs) when sneaker males took part in spawning (Watters 2005). Females appeared to prefer mating with sneaker males to avoid direct costs of mating that are imposed on them when spawning with aggressive guarder males (Watters 2005). Similarly, female bluegill sunfish, *Lepomis macrochirus*, laid three times more eggs when sneaker males participated in spawning (Fu et al. 2001). In the present study we found no evidence that females were able to avoid matings with unconstrained dominant males, although it was notable that subordinate males were often successful in fertilizing a proportion of eggs through sneaking. Whether female behavior played a role in influencing the success of subordinate males cannot be determined from our experimental design, although previous studies in the related *R. amarus* suggests it can (Smith and Reichard 2005; Smith et al. 2007). The current study provided no evidence for variation in mean clutch size among experiments 1–3; larger clutches were not produced when groups of males were able to participate in matings (experiment 3) compared to when only individual males were able to fertilize eggs (experiments 1 and 2).

Strong mate choice coupled with weak congruence among females in their choice of males in experiments 1 and 2 suggests a role for nonadditive genetic benefits in female mate choice, rather than a strong effect of additive benefits; females showed significant preferences for particular males, but were not always consistent in preferring the same males. Notably, eggs fertilized by preferred males showed significantly higher survival to independence (experiment 4), providing evidence for a fitness benefit to female mate choice.

There is an ongoing controversy over the importance of additive benefits of female mate choice (Kirkpatrick and Barton 1997; Møller and Alatalo 1999; Head et al. 2005), and only a limited number of studies provide substantial evidence for an effect (e.g., three-spined stickleback, *Gasterosteus aculeatus*, Barber et al. 2001; gray tree frog, *Hyla versicolor*, Welch et al. 1998). Zeh and Zeh (1996) argued that genetic incompatibilities between potential mates, arising from intragenomic conflict, could substantially decrease fertilization rate and offspring viability, which could generate strong selection for mate choice based on compatibility. In a study of the Alpine whitefish, *Coregonus* sp., Wedekind et al. (2001) demonstrated that offspring sired by males with the most extensive breeding ornamentation had higher survival rates following bacterial infection at the egg stage regardless of female identity, suggesting additive genetic benefits to mating. However, offspring mortality arising from developmental problems appeared related to specific male–female combinations, suggesting incompatibilities between parental haplotypes (Wedekind et al. 2001). In the related Arctic charr (*Salvelinus alpinus*), the fertilization success of sires correlated positively with their genetic similarity, measured using microsatellites, with dams (Liljedal et al. 2008); a result consistent with a role for genetic compatibility.

Recent work suggests that females may be capable of choosing genetically compatible mates through olfactory cues based on MHC-specific odors (Jordan and Bruford 1998); a substantial body of empirical evidence for MHC-based mate choice has accumulated (Neff and Pitcher 2005; Piertney and Oliver 2006). In this case mate choice operates through a self-referential process involving detection of MHC peptide ligands (Milinski et al. 2005). MHC preferences may manifest themselves as pre-mating behavioral decisions but also as postmating fertilization or developmental incompatibilities (Tregenza and Wedell 2000; Skarstein et al. 2005).

The mechanism of female mate choice in rose bitterling is not known. Olfactory cues play a key role in mate choice in many fish, including poeciliids (McLennan and Ryan 1997) and zebrafish (Spence et al. 2008), and there is evidence that the same is true in bitterling (M. Agbali, unpubl. data). Chemical cues in fish may be released from specialized exocrine glands, or if water-soluble compounds are released, from the gills or in the urine (Rosenthal and Lobel 2006). The release of chemical compounds by fish is often associated with fin or body movements, because the diffusion of compounds in water is relatively slow (Atema 1996). In the case of bitterling, male courtship behavior involves the male swimming in front of the female and undulating his body, and particularly the anal and tail fin, at high frequency and low amplitude (Smith et al. 2004), which may assist in directing male odor toward courted females. Female mate choice correlated significantly with male courtship rate in the present study, an observation that is consistent with previous field and laboratory studies on bitterling (Smith et al. 2002; Reichard et al. 2005). In the case of experiment 4, the correlation between male reproductive success and courtship was a consequence of male dominance; dominant males prevented subordinate rivals from courting or mating, except through sneaking, resulting in a strong relationship, but not one derived from female mate choice. Courtship behavior in bitterling may be associated with the release of olfactory cues by males, possibly in the urine (M. Agbali, unpubl. data). Thus, one hypothesis is that females may choose preferred mates through olfactory cues based on MHC-specific odors. Ongoing work will explore the role of MHC gene compatibility in offspring fitness, and the role of MHC-specific odors in mate choice decisions of bitterling.

The findings of this study suggest differences in the outcome of sexual selection for dominant and subordinate males, depending on the social or environmental context in which they compete. For example, in an environment in which male contests for mating opportunities are limited, and females are able to exercise mate choice without constraint, otherwise subordinate males that court vigorously are predicted to enjoy high reproductive success. In contrast, in an environment that results in strong intrasexual selection the reverse will be true with dominant males able to largely monopolize matings. The bitterling offers an excellent model for these studies; previous work has shown that bitterling occur in lakes in which their population size varies 90-fold and mussel abundance 500-fold (Smith et al. 2000a). Consequently female choice may be more important in populations in which bitterling densities are low and mussel densities are high; all male bitterling could potentially hold territories and interference between males is predicted to be limited. In contrast, when population densities are high and mussel abundances are low, conditions will favor competition for spawning sites through male dominance.

Male nuptial coloration weakly predicted male dominance. In experiment 2, in which males were exposed to rivals but unable to interact directly, the extent of redness in the iris correlated positively with male dominance, and in experiment 4, in which males were isolated, dominance matched the degree of tail spot redness. In contrast, in experiments 1 (males isolated) and 3 (males able to interact fully) neither iris nor tail spot redness corresponded with dominance. However, although male coloration appeared to function, albeit to a limited extent, in intrasexual competition, there was no evidence that it played a role in intersexual selection; male color failed to predict female mate preference in any experiment. These findings match those for a study on a related species, the European bitterling, *R. amarus*. Reichard et al. (2005) found no effect of the intensity of male eye or fin color on female mate choice, although the intensity of eye color predicted male reproductive success in a competitive environment. Similarly, Smith et al. (2002) found the extent of eye redness to be greater among dominant males under natural conditions of *R. amarus*. Candolin and Reynolds (2001) did detect a significant effect of the intensity of fin color on female spawning decisions, although this finding has never been repeated. Thus, although the extent and intensity of male coloration may have some limited function as a badge of status (Andersson 1994), further research will be needed to establish the functional role of male coloration in bitterling fish.

In conclusion, it was demonstrated that intersexual selection through female mate choice and intrasexual selection through aggressive male contests do not correspond in *R. ocellatus*; females appear to suffer a net fitness loss as a consequence of intrasexual selection suggesting an intersexual conflict. The lack of strong congruence in female preference for males suggests a significant role for mate compatibility in mate choice, possibly mediated through MHC-specific odors.

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