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Sexual selection for male dominance reduces opportunities for female mate choice in the European bitterling (*Rhodeus sericeus*)

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Abstract

Sexual selection involves two main mechanisms: intrasexual competition for mates and intersexual mate choice. We experimentally separated intrasexual (male–male interference competition) and intersexual (female choice) components of sexual selection in a freshwater fish, the European bitterling (*Rhodeus sericeus*). We compared the roles of multiple morphological and behavioural traits in male success in both components of sexual competition, and their relation to male reproductive success, measured as paternity of offspring. Body size was important for both female choice and male–male competition, though females also preferred males that courted more vigorously. However, dominant males often monopolized females regardless of female preference. Subordinate males were not excluded from reproduction and sired some offspring, possibly through sneaked ejaculations. Male dominance and a greater intensity of carotenoid-based red colouration in their iris were the best predictors of male reproductive success. The extent of red iris colouration and parasite load did not have significant effects on female choice, male dominance or male reproductive success. No effect of parasite load on the expression of red eye colouration was detected, though this may have been due to low parasite prevalence in males overall. In conclusion, we showed that even though larger body size was favoured in both intersexual and intrasexual selection, male–male interference competition reduced opportunities for female choice. Females, despite being choosy, had limited control over the paternity of their offspring. Our study highlights the need for reliable measures of male reproductive success in studies of sexual selection.

Keywords: body size, carotenoid colouration, mate preference, mating tactics, parasite load, territoriality

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Introduction

Sexual selection arises from intrasexual variance in reproductive success and is typically higher among males because of differences in gamete allocation between the sexes. There are two main mechanisms by which sexual selection can occur: intrasexual competition for mates (typically male–male interference competition) and intersexual mate choice (typically female choice). Under male–male interference competition, males actively compete for access to females or resources that are necessary to attract females. Under female choice, males compete to be chosen by females who base their preferences on direct or indirect (or both) signals of male quality (Darwin 1871; Andersson 1994). Some sexually selected traits appear to be based mainly on one of these two components of sexual selection. For example, horns in many ungulates and beetles serve as weapons in male combat, while elaborate signals and

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displays, such as the ornate train of peafowl, appear to be driven solely by female choice (Andersson 1994). However, other traits may play a role in both intra- and intersexual selection simultaneously, with different selection pressures arising from each component (Moore & Moore 1999; Maynard-Smith & Harper 2003).

Both male–male competition and female choice have been the subjects of a considerable research effort, whereas their relative contribution to sexual selection is poorly understood (Maynard-Smith & Harper 2003). This oversight is surprising, because many male traits may be important for both intra- and intersexual selection, either acting in concert or in opposition (Qvarnström & Forsgren 1998; Moore & Moore 1999). For example, in many populations of the three-spined stickleback (*Gasterosteus aculeatus*), the red nuptial colouration of males is a characteristic of dominant individuals that win fights (De Fraipont *et al*. 1993), and females prefer to mate with redder males (Milinski & Bakker 1990). The variation in redness increases under male–male competition and this facilitates female choice (Candolin 2001). In contrast, conflicting selection pressures are involved in the production of social pheromones by the male speckled cockroach (*Nauphoeta cinerea*). Optimal pheromone signalling differs between male–male competition and female attraction, and male cockroaches with a pheromone level that makes them superior in establishing male dominance are not preferred by females (Moore & Moore 1999).

Sexual selection does not conclude at mating. Sperm competition (postcopulatory male–male competition, Parker 1970) and postcopulatory cryptic female choice (Eberhard 1996) may bias the paternity of offspring and consequently can play an important role in the strength of sexual selection. Therefore, behavioural predictions of male reproductive success may often be inaccurate (Gemmell *et al*. 2001; Double & Cockburn 2003; Say *et al*. 2003). Paternity estimates are reliable measures of male success under sexual selection, having the advantage of showing how the outcome of intra- and intersexual selection translates to reproductive success. To our knowledge, offspring paternity analysis has not yet been applied to experimental studies aimed at addressing the success of both these components of sexual selection simultaneously.

Here, we attempt to disentangle the outcome of intra- and intersexual selection in the European bitterling (*Rhodeus sericeus*), a small cyprinid fish with a resource-based mating system. We investigated how multiple phenotypic (both morphological and behavioural) traits are related to either intra or intersexual selection in the bitterling, and how they translate to reproductive success estimated in terms of the paternity of the offspring produced.

In bitterling, both intra- and intersexual selection may be important. During the breeding season, males develop red carotenoid-based nuptial colouration 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 actively court females and lead them to mussels in their territories. Females swim in large shoals and receptive females individually inspect several males and their mussels before spawning. Female oviposition decisions are based on both male and mussel quality (Candolin & Reynolds 2001; Smith *et al*. 2002). If a female chooses to spawn, she quickly inserts her ovipositor into the exhalant siphon of a mussel and deposits one to six (typically three) eggs. Females may have control over the number of eggs they deposit during a spawning, varying the number according to the quality of the mussel (Mills & Reynolds 2002) and male (Smith & Reichard, unpublished).

Males release sperm over the inhalant siphon of the mussel and fertilization occurs inside the gill chamber. Sneaking behaviour, in which a rival male (often an adjacent territory holder or a male that does not possess a territory) releases his sperm over a territory holder's mussel, is common in bitterling (Smith *et al*. 2002, 2003; Reichard *et al*. 2004a). Male mating behaviour is largely opportunistic and there is no evidence of a fixed morphological or genetic distinction between territorial and sneaking males. Females spawn in several bouts lasting 1 or 2 days and consisting of at least five independent spawnings each day. For a review of bitterling reproductive biology, see Smith *et al*. (2004).

To partition the effects of behavioural and morphological traits on female choice and male–male interference competition, we conducted an experiment with pairs of males. First, we tested female preferences when males were prevented from competing. We then allowed males to establish dominance and spawn with females. Finally, we used genetic markers to assign the reproductive success of each male and relate it to male traits, female preference and male dominance.

If both components of sexual selection operate together, males bearing those traits favoured under both forms of sexual selection are predicted to have high reproductive success, resulting in high reproductive skew among males in the population. However, if there is a conflict in these two components of sexual selection, with female choice and male–male competition favouring different male phenotypic traits, then selection pressure on specific male traits may be weaker, thereby maintaining genetic variation in a broader range of male traits. The extent of this variation will then be determined by the relative contribution of male dominance and female choice to male reproductive success; variation will be greatest if both components contribute to male success and lowest if one component is the main determinant.

Materials and methods

Experimental set-up

Fish used in experiments were captured in the River Kyjovka in the southeast of the Czech Republic in late April 2003 using a DC electroshocker modified to catch small fish with minimal stress and injury. Fish were transported to a large outdoor concrete pool (12.4 × 6.0 m, water depth, 0.6 m) at the Institute of Vertebrate Biology (IVB), Brno, Czech Republic. The pool was equipped with artificial vegetation and mussels in sand-filled flowerpots. Fish grazed on a carpet of algae that established on the walls and floor of the pool and were additionally fed twice each day on frozen chironomid larvae. Mussels (*Anodonta anatina*) used in the experiment were collected from a small lake adjacent to the River Kyjovka in early April, before the onset of bitterling spawning. Mussels were stored in large shaded outdoor containers at the IVB with a thick layer of sand and continuous aeration and fed phytoplankton daily.

Experiments were conducted in seven aquaria measuring 75 × 40 × 40 cm with a layer of sand and which were continuously aerated. Experimental aquaria were isolated using opaque barriers so that fish in adjacent aquaria could not interact. Aquaria contained two transparent glass boxes (35 × 11 × 11 cm) that were positioned in the left and right back corners adjacent to sand-filled flowerpots, each containing a single mussel. Experimental mussels were haphazardly selected from a stock of similar-sized mussels (mean ± SE shell size, 82 ± 0.9 mm) to avoid any possible effects of mussel size on fish behaviour. Each aquarium was equipped with artificial vegetation that divided aquaria into two identical sections and prevented visual interactions between males during female choice trials when males were constrained. Aquaria were held under a natural light cycle and water temperature matched natural variations (17–20 °C). When experimental fish were not present in aquaria, an internal filter was used to maintain water quality but was disconnected when experiments were taking place. All experimental replicates were carried out between 20 May and 2 June 2003.

Experimental protocol

Experimental fish were caught in the pool by a diver using a hand net and immediately transported to the aquarium room. Two randomly selected males were introduced into the glass boxes in corners of the aquarium after a fin clip from the caudal fin was taken (a tip of the lower or upper lobe for different males in each pair, respectively, to distinguish their identity later in the experiment) and fixed in ethanol. Males were allowed to settle for 30 min; males swam naturally and did not show any signs of distress during this time. After 30 min, a receptive female with an extended ovipositor was gently released into the aquarium and after another 30-min, behavioural recording started. Each minute, within a 30 min observation period, the following behaviours were recorded: male courtship (male quivers his body, exposing his lateral side) and female response (female approach to the male). Female presence next to a constrained male in closed compartments is a standard measure of female preference in fishes. In species where readiness to spawn may be unambiguously assessed (including bitterling), this has been shown as a reliable indicator of female willingness to spawn with a particular male (reviewed in Gonçalves & Oliveira 2003). Following these trials, the males were gently released from the boxes and the fish were left in aquaria for 20 h. During that time all aquaria were observed on three occasions and dominance between males was recorded. Dominant males were recognized by their overt aggression to the other male and territorial defence of the mussels. Males were individually identified by their unique fin clip.

At the end of each trial, the fish were captured and a fin clip was taken from the female's caudal fin and stored in ethanol. Males were captured and the left lateral side was photographed within 3 min. All fish were measured for body length (from tip of the mouth to the base of the tail fin). Male body depth (perpendicular height from the front base of the dorsal fin, i.e. the highest point on the body) was measured from scaled photographs and regressed against body length. The resulting residual body depth was used in all subsequent analyses. Females were released back into the pool and male pairs were held in 15 L netted containers inside the pool for subsequent parasite screening. A total of 26 replicates were completed that we considered sufficient to detect significant differences in female preference and male dominance in respect to male traits based on our previous studies (reviewed in Smith *et al*. 2004). No fish was used more than once in the experiment.

Experimental mussels were isolated and after six days bitterling embryos were dissected from mussel gills. Embryos were not recovered from all mussels, which commonly eject eggs and developing embryos. Consequently, only 14 replicates have been analysed for paternity. The mortality rate of bitterling embryos is naturally high, and our experimental design did not allow us to differentiate whether the lack of embryos in mussels resulted from a failure of fish to spawn or through embryo mortality. However, all experimental females possessed extended ovipositors and were capable of spawning. A comparison with mortality rates in previous experiments (Reichard *et al*. 2004b) indicates that the observed variability in the number of embryos and absence of offspring in some replicates may result exclusively from embryo mortality, arising from eggs ejections (Mills & Reynolds 2002) and failure of fertilization (Smith & Reichard, unpublished).

Analysis of male colouration

Male bitterling develop red colouration on their anal fin, ventral part of the body and iris of the eye. Red colouration of the anal fin and body was not estimated because, unlike the pigment of the eye, it is under neuronal control and changes rapidly when a fish is captured. The colouration of males was quantified following the methods of Barber *et al*. (2000), Candolin & Reynolds (2001) and Smith *et al*. (2002). Males were photographed in standardized conditions with a scale (a rule marked in 1 mm increments) to serve as a reference during body depth analysis. The same professional lab developed all the images, which were digitized for further analysis. MICROIMAGE for Windows 4.0.0 was used to measure the extent of red colour in the iris, both as a proportion of the total iris area (pupil excluded; P) and the total red area (T, in mm^2). In ADOBE PHOTOSHOP 7.0, five pixels from the red area of the iris were randomly selected and their red index (R, proportion of the brightness of the red component to the sum of red, blue and green component values) was calculated.

Resulting values (P, T, R) were subjected to principal component analysis (McGraw & Ardia 2003). The first component (PC1, eigenvalue = 2.07) explained 63% of variation and was related to the red extent (factor loadings $P = 0.94$ $T = 0.96$, $R = 0.52$) while the second component (PC2, eigenvalue = 0.85, 28%) was related to variability in red intensity (factor loadings $P = -0.30$ $T = -0.17$, $R = 0.86$). The contribution of the third component (PC3, eigenvalue = 0.08, 3%) was negligible. In further analyses, PC1 is considered as a measure of the extent of eye redness, while PC2 refers to the intensity of redness.

Paternity analysis

DNA from fin samples or embryos with detached yolk sac was isolated after Proteinase K digestion by phenol–chloroform extraction using 2-mL Phase-lock-gel tubes (Eppendorf). In one embryo (family 23), DNA extraction was not successful, even after three replications and this embryo has been excluded from further analyses. Two putative sires, the female, and embryos from 15 replicates of the experiment were genotyped. In two replicates, mussels contained several embryos from spawnings that occurred before the experiment began. These embryos were recognized by their older developmental stage and lack of maternal alleles. One mussel contained a mixture of experimental and older embryos, while the other contained solely extra-experimental embryos. In 11 replicates, no embryos were recovered from mussels. This reduction in replication decreased the power of our analysis of reproductive success.

We identified the multilocus genotype on the basis of eight highly variable microsatellite loci: *Rser01–06*, *Rser08*, and *Rser10* (Dawson *et al*. 2003). Microsatellite loci were amplified via two multiplex polymerase chain reactions (PCRs) in a PCR machine (Robocycler, Stratagene), with four pairs in each reaction. Forward primers were labelled by a fluorescent dye (FAM, HEX, or TET) and the final concentration of each primer in the reaction mixture was 0.1 μM, except for *Rser04* primers that were used in 0.2 μM concentration. The 20-μL reaction volume contained primers for four loci, approximately 100 ng of genomic DNA, 1 unit of *Taq* polymerase (Fermentas), 1 × Mg-free reaction buffer (Fermentas), 0.2 mM dNTPs, and 3 mM $MgCl_2$. The thermal profile of reactions consisted of initial 3 min denaturation at 94 °C, followed by 30 cycles of 94 °C for 40 s, 61 °C for 30 s, and 72 °C for 60 s, concluding with a final 7 min extension at 72 °C. The PCR products (0.8 μL) were added to a denaturing mixture of size standard (Genescan, TAMRA 500 or ROX 400, Applied Biosystems) and formamide. After 5 min denaturation in 96 °C and 2 min cooling on ice, the mix was loaded on the ABI Prism 310 Genetic Analyser (Applied Biosystems) for separation and detection. The length of the DNA fragments was analysed using GENESCAN software (Applied Biosystems).

The observed heterozygosity enabled paternity assignment by an exclusion of incompatible paternal genotype for all but a single embryo. This unassigned embryo (family 2) was excluded from further analyses. Genotyping was not repeated and therefore we could not estimate the level of genotyping error. However, low locus polymorphism at most loci and a low number of candidate parents (maternal genotype known, two putative sires) reduced the probability of false paternity assignment (Hoffman & Amos 2005). A null allele, reported at *Rser04* in our previous study (Reichard *et al*. 2004b), has been identified as alleles of > 400 bp that could not have been scored with the equipment used in a previous study. For details on microsatellite loci used, see Table 1.

Table 1 Estimates of genetic parameters of the eight microsatellite loci used. Observed and expected heterozygosities and deviation from Hardy–Weinberg equilibrium were calculated in CERVUS 2.0 (Marshall *et al*. 1998), exclusively from genotypes of adult fish

Locus name	N_{ad}	N_a	Fragment size	H_O	H_E	HW
Rser01	45	5	175–183	0.36	0.35	ns
Rser02	45	2	177–179	0.33	0.31	ns
Rser03	45	4	224–232	0.67	0.65	ns
Rser04	45	30	247–454	0.84	0.87	ns
Rser05	41	3	228–236	0.49	0.50	ns
Rser06	45	5	125–133	0.44	0.44	ns
Rser08	45	6	188–205	0.38	0.37	ns
Rser10	45	3	197–201	0.44	0.51	ns

N_{ad}, number of parental fish; N_a, number of alleles at a given locus; H_O, observed heterozygosity; H_E, expected heterozygosity; HW, deviation from Hardy–Weinberg equilibrium (ns, nonsignificant).

Parasite screening

After all replicates were completed, males from replicates that produced offspring were examined for the presence of metazoan parasites. Fish were humanely killed by cutting the spine at the base of the skull using a pair of sharp scissors on a wet Petri dish. Fish were dissected and parasites were removed from the host tissue (fins, body surface, gills, brain, eyes, internal organs, muscles) according to an established protocol (Ergens & Lom 1970), and fixed according to parasite taxon. All parasites were subsequently identified using a light microscope equipped with phase-contrast, differential interference contrast, and digital image analysis.

Three values were calculated from parasitological data. Parasite load refers to the total number of metazoan parasites. Parasite species richness is the number of metazoan parasite taxa found on a given individual. Number of *Diplostomum* refers to the number of individual *Diplostomum* cf. *spathaceum* metacercariae (larval stage) on the eye lenses. The former two measures were used throughout the analysis. The number of *Diplostomum* was only tested for a correlation with the eye redness values. Only males that produced offspring and one additional pair were screened for parasites for ethical and logistical constraints. At least one male from three male pairs escaped from the netted containers within the pool and males from these pairs were not screened. These escapes resulted in 12 complete replicates.

Statistical analyses

Female mate preference was calculated from the number of positive female responses to confined males during a 30-min choice test (giving values between 0 and 30 for every male, maximum score of 30) and had a binomial distribution; each male was either preferred (higher female response score) or nonpreferred (lower female response score). Dominance between males (see previous discussion for characterization) was scored three times over a 20-h period. Two measures of reproductive success were used in our analyses. To split males into two groups for the paired comparisons, males that sired most of the offspring within a replicate were designated as successful, their rival as unsuccessful. Unsuccessful males did not sire any offspring except for one male that sired one of 16 (i.e. 6%) embryos in his replicate. Two replicates were not considered for univariate analyses because both males fathered equal number of offspring (Fig. 1). In multivariate analysis, absolute number of offspring sired by a particular male was used.

Where male traits were not normally distributed, they were log, arcsine or square-root transformed to meet assumptions of normality. Paired *t*-tests or Wilcoxon paired tests (when variable did not respond to transformation) where used to compare preferred and nonpreferred (female preference), dominant and subordinate (male dominance), and successful and unsuccessful (reproductive success) males. Following the recent recommendation of Nakagawa (2004), Bonferroni corrections were not applied and instead, a measure of effect size (Cohen's *d*) was estimated. This index measures the magnitude of a treatment effect as the standardized difference between two means by comparing the overlap in the distribution between the two data sets independently of sample size. To avoid its overestimation, arising from a paired design, we calculated Cohen's *d* from original mean values and standard deviations rather than from the *t*-test value.

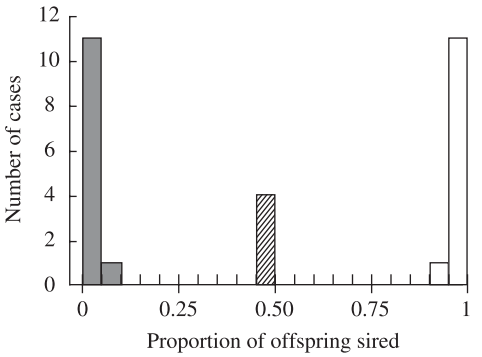

Fig. 1 Proportions of offspring sired by individual males. Males that were designated as successful (white bar), unsuccessful (dark bar), and those that were not used (hatched bar) in paired comparisons are indicated.

To account for intercorrelations between male traits, stepwise regression models were used to detect which male traits best explained variance in female preference, male dominance, and male reproductive success. As a result of incomplete design for parasite screening, measures of parasite load were not included in these analyses. Logistic regressions with a binomial distribution (type 1 sequential likelihood-ratio test) were used for female preference and male dominance. For analysis of male reproductive success, males were arbitrarily named A and B, and male B paternity (P_B, difference between the number of offspring sired by male B and male A) was calculated. P_B was then related to the differences in behavioural and morphological traits of the two putative sires (Evans *et al*. 2003), and a generalized linear model regression (GLMR) with normal distribution was employed. All statistical analyses were conducted using STATISTICA 6.0.

Results

Female choice

Females responded to male courtship in all replicates. The regression model revealed that the vigour of male

Table 2 Results of stepwise regression analyses of the contribution of male traits to female preference, male dominance (both generalized linear model regression with binomial distribution, type 1 sequential likelihood-ratio test), and reproductive success (GLMR with normal distribution)

	Female preference		Male dominance		Reproductive success	
	χ^2	*P*	χ^2	*P*	*F*	*P*
Standard length	4.31	0.038	3.85	0.050	0.12	0.736
Residual body depth	1.95	0.162	0.50	0.481	0.36	0.561
Extend of red PC1	1.44	0.230	0.26	0.612	1.37	0.269
Red intensity PC2	0.32	0.570	2.96	0.085	7.31	0.021
Courtship	5.61	0.018	0.37	0.543	2.40	0.153
Female preference			0.01	0.954	0.93	0.357
Male dominance					14.00	0.003

courtship was the best predictor of female preference, followed by male body size (GLMR with binomial distribution; Table 2). Univariate analyses corroborated results from the regression model; females spent significantly more time next to males that courted more vigorously (Wilcoxon paired test, $T = 10.0$, $n = 26$, $P = 0.041$, $d = 0.44$) and were larger (paired *t*-test, $t_{25} = 2.12$, $P = 0.045$, $d = 0.43$) than their rivals. Courtship vigour and body length were not correlated (Spearman rank correlation, $r_s = 0.14$, $n = 52$, $P = 0.320$). Residual body depth (paired *t*-test, $t_{25} = 1.05$, $P = 0.303$, $d = 0.29$), the extent of red colour in the eye (paired *t*-test, $t_{25} = 1.40$, $P = 0.173$, $d = 0.41$), and intensity of eye redness (paired *t*-test, $t_{25} = 0.51$, $P = 0.613$, $d = 0.10$) had no effect on female preference. The effects of total parasite load (paired *t*-test, $t_{11} = 1.77$, $P = 0.105$, $d = 0.83$) and parasite species richness (Wilcoxon paired test, $T = 6.0$, $n = 12$, $P = 0.051$, $d = 1.06$) were not statistically significant.

Male dominance

An unambiguous dominance between males (consistent dominance status over the three inspections) was established in 21 (81%) replicates. In the other replicates, dominance status varied among independent inspections or could not be clearly estimated within a period of 3 min for at least one observation. When clear dominance was established, the dominant male always controlled the mussels. Preferred males established dominance in 13 cases and female response was not related to dominance rank (paired *t*-test, $t_{25} = 1.53$, $P = 0.141$, $d = 0.64$). Male body size was a single explanatory variable that described male dominance in a GLMR with a binomial distribution (Table 2). Univariate tests supported the outcome of the multivariate analysis; dominant males were larger than their rivals (paired *t*-test, $t_{20} = 2.19$, $P = 0.040$, $d = 0.52$). Residual body depth (paired *t*-test, $t_{20} = 0.32$, $P = 0.756$, $d = 0.01$), extent of red in the eye (paired *t*-test, $t_{20} = 0.15$, $P = 0.882$, $d = 0.05$), intensity of eye redness (paired *t*-test, $t_{25} = 1.44$, $P = 0.166$, $d = 0.38$), courtship vigour (Wilcoxon paired test, $T = 7.0$, $n = 21$, $P = 0.237$, $d = 0.64$), total parasite load (paired *t*-test, $t_{11} = 1.18$, $P = 0.262$, $d = 0.57$) and parasite species richness (Wilcoxon paired test, $T = 6.0$, $n = 12$, $P = 0.051$, $d = 1.35$) had no statistically significant effect on dominance.

Table 3 Number of recovered embryos that were successfully genotyped (given separately for individual mussels), and the number and proportion (in percentage) of the offspring sired by the dominant male. In two cases, when dominance could not be unambiguously determined, the number of embryos sired by individual males is given in parentheses

Family	*N* of genotyped embryos	Paternity of dominant male	(in %)
2	3 + 0	0	0
3	5 + 2	7	100
4	8 + 0	(4 + 4)*	(50 + 50)
9	23 + 0	23	100
14	2 + 0	0	0
16	2 + 0	2	100
17	1 + 0	1	100
19	11 + 0	11	100
20	8 + 5	13	100
21	3 + 1	2	50
22	16 + 0	15	94
23	9 + 8	17	100
25	6 + 1	0	0
26	3 + 0	(3 + 0)*	(100 + 0)

*no dominance.

Reproductive success

Experimental males shared paternity of offspring in three of 14 (21%) replicates that yielded offspring. In two replicates (with four and eight embryos), both males achieved the same reproductive success. In one replicate the subordinate male sired one of 14 embryos (6%). In five replicates, embryos were recovered from both mussels; in four cases, a single male sired all the embryos in the replicate. In one replicate (family 21), one mussel yielded a multiply sired clutch of three eggs and the second contained a single embryo; both males sired two embryos in that replicate (Table 3).

A regression model (GLMR with normal distribution) revealed that male dominance, followed by intensity of red colouration, yielded males the greatest reproductive success (final model: $R^2 = 0.62$, $F_{2,10} = 8.22$, $P = 0.008$; Table 2). Univariate tests confirmed that dominant males were more successful than subordinate males (binomial test, $N = 12$, $P = 0.023$). Within male dyads, larger males (paired t-test, $t_{11} = 3.97$, $P = 0.002$, $d = 0.99$) attained greater reproductive success than rivals. Female choice alone did not affect male reproductive success (paired t-test, $t_{11} = 0.56$, $P = 0.586$, $d = 0.30$). Similarly, no effect was found for residual body depth (paired t-test, $t_{11} = 0.86$, $P = 0.407$, $d = 0.36$), extent of red colour in the eye (paired t-test, $t_{11} = 0.16$, $P = 0.874$, $d = 0.08$), eye redness intensity (paired t-test, $t_{11} = 0.95$, $P = 0.365$, $d = 0.34$), parasite load (paired t-test, $t_9 = 0.37$, $P = 0.722$, $d = 0.20$), and parasite species richness (Wilcoxon paired test, $T = 9$, $n = 10$, $P = 0.398$, $d = 0.47$).

Parasite screening

Parasite load ranged between 0 and 5 (mean 2.0 ± 0.3 SE) parasites on each fish with a maximum of three taxa (mean 1.5 ± 0.2 SE) per fish. The highest prevalence (46% of all fish were infected) was found for metacercariae of *Diplostomum* cf. *spathaceum* parasitizing the eye. Other trematode metacercariae (*Clinostomum complanatum*, *Metagonimus yokogawai*, *Metorchis intermedius*, and *Posthodiplostomum cuticola*), monogeneans (*Paradiplozoon homoion*, *Gyrodactylus rhodei*) and nematodes (*Pseudocapillaria tomentosa*) showed a low prevalence. No correlation between measures of parasite load (total parasite load, number of *Diplostomum* in eyes, parasite species richness) and extent or intensity of red colour in the eye was detected (Spearman rank correlation, $N = 24$, all $P > 0.17$).

Discussion

We found that the dominance status of male bitterling was the most important determinant of their reproductive success, measured as both the number and the proportion of offspring a male sired. Although the same morphological trait (larger body length) was favoured by both intersexual and intrasexual components of sexual selection, the vigour of courtship had the strongest effect on female preference, but was not related to male dominance. Therefore, male–male interference competition reduced the opportunities for female choice.

Male traits

Among morphological traits, body size was an important factor in both male–male competition and female choice. Furthermore, males with the most intense red colouration in their iris sired more offspring than other males (Table 2). In contrast, the extent of red area in the eye and parasite load did not affect female choice, male dominance or male reproductive success. We also detected no effect of parasite load on the expression of carotenoid-based colouration, though there was an overall low prevalence of parasites in the fish in our experiment.

The fact that body size played an important role in both male–male competition and female choice is common. A comparison across several taxa showed that large body size in males is usually selected in male contests, and less often by female choice or a combination of the two (Andersson 1994). This corresponds with our previous finding that larger male bitterling are more likely to establish territories in a situation when male abundance exceeds the number of mussels available (Reichard *et al*. 2004b).

Carotenoid-based colouration has been demonstrated to provide information on several aspects of male quality, such as foraging ability (Endler 1980), immunocompetence (McGraw & Ardia 2003), nest defence during parental care (McKinnon 1996), or resistance to parasites (Houde & Torio 1992; Barber *et al*. 2001), and is used as a cue in female choice for a number of bird and fish species (reviewed in Andersson 1994; Olson & Owens 1998). However, female preference for brightly coloured males may vary among populations, being affected by local conditions (Endler & Houde 1995) or trait variability among individual males (Braithwaite & Barber 2000). In two populations of the three-spined stickleback, females chose the redder male if the difference between males was high, but often did not if two males had a similar extent of red colour (Braithwaite & Barber 2000; Candolin 2001). Unlike birds, red colouration on the body and fins of fishes, including bitterling, can change rapidly (Wiepkema 1961; De Fraipont *et al*. 1993; Candolin 2001; Candolin & Reynolds 2001). In contrast, red pigment in the bitterling eye appears to be more stable (Kim *et al*. 1999; Smith *et al*. 2004; M. Reichard, personal observation) and, consequently, we used eye colouration in our analyses to prevent any effect of fish handling on colour expression.

We did not detect any significant effect of male eye redness on female choice or male dominance, though multivariate analysis revealed that males that become dominant over their rivals tended to have a higher intensity of red colour in the eye. This trend appeared to be statistically significant only as a direct effect on absolute reproductive success (Table 2). One explanation for the lack of a significant relationship between eye redness measures and female choice may be related to the fact that carotenoids may not be rare in the diet of bitterling, which feed predominantly on green and red algae (Przybylski 1996). Therefore, the expression of carotenoids as red pigments may not be as costly as in other species (Olson & Owens 1998). Second, red colour intensity may function as a badge

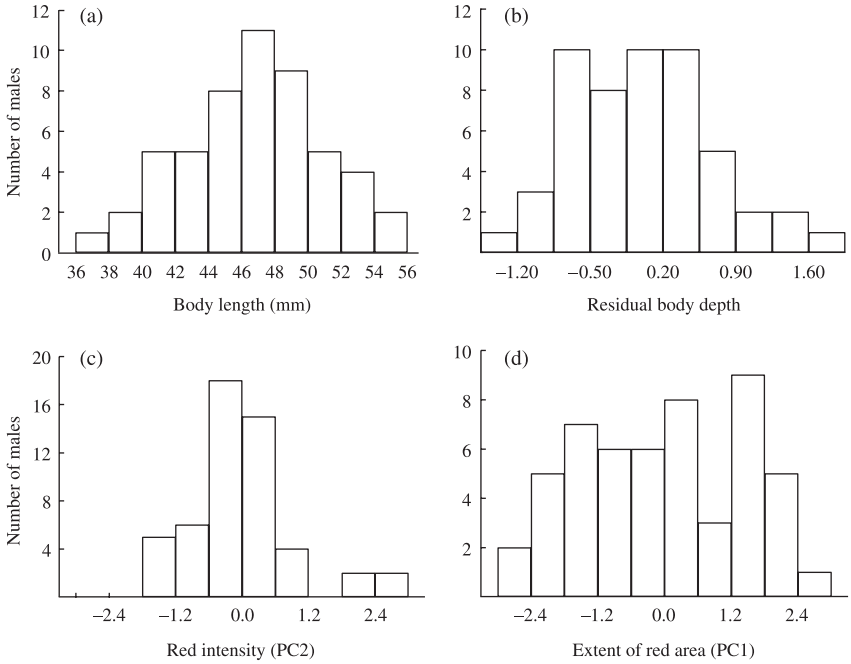

Fig. 2 Histograms showing the distribution of male morphological traits: (a) body length, (b) residual body depth, (c) intensity of red colour in eye iris, and (d) extent of eye redness.

of dominance status. Red colouration often signals the level of male aggression and dominance (for example, in fish: Evans & Norris 1996; Candolin 2001; in birds: Mateos & Carranza 1997; Pryke *et al*. 2002). Thus, the expression of red colour in bitterling might signal success in male–male competition rather than foraging ability, though this hypothesis remains to be tested.

Our experimental design did not include measurement of eye redness before trials (to minimize stress). Consequently, we could not test whether eye redness intensity predicted the outcome of subsequent male–male competition, or whether redness intensity changed during trials. Candolin (2001) showed that male three-spined sticklebacks adjust the redness of their body colouration according to their dominance rank and level of aggression. However, Le Comber (2004) showed that, although male three-spined stickleback redness varied during mating trials, the rank order of male redness was unaltered. If eye redness played no role in female choice, our results indicate that some male traits that are considered to be driven by intersexual selection may instead have a function in intrasexual competition (Rowland 1994).

In our experiment, males were randomly selected from the experimental population rather than taken from *a priori* categories (e.g. red and dull, small and large). We believe that this approach better fits the natural situation and helped us to recognize relative contributions of multiple uncontrolled traits. However, such an approach also unavoidably decreases the power to detect strong biases towards extreme phenotypes. We do not discount the possibility that females might show a preference for redder males if compared to a markedly less red opponent. Nevertheless, such a decision probably occurs rarely in natural situations if the trait distribution is normal rather than bimodal, which is the case for male traits in our analyses (Fig. 2).

The roles of intra- and intersexual selection

Our study demonstrated that female mate choice was undermined by the outcome of male–male interference competition. Males that initially courted females more vigorously were preferred, but this preference had no effect on their reproductive success. Instead, males that were dominant over their rivals during male–male competition sired most offspring.

Female choice for courtship vigour overrides preference for male body size in experiments that exclude male–male competition and is consistent across bitterling populations (Candolin & Reynolds 2001; Smith *et al*. 2002) and in other taxa (Kotiaho *et al*. 2001). However, female preference may be seriously impaired under male–male competition. We showed that preferred males became dominant only in 50% of replicates and dominant but not necessarily preferred males monopolized most matings. This result raises an apparent paradox of how female choice can be maintained in the face of male dominance.

Persistence of female choice may result from the fact that under natural conditions, not all matings involve male–male competition. In previous field (Smith *et al*. 2002) and mesocosm (Reichard *et al*. 2004b) studies, 32% (n = 69) and 17% (n = 52) of spawnings were without rival male interference. Furthermore, female choice may be still more powerful than male dominance if females

choose traits with high heritability and which confer high offspring fitness (Drickamer *et al*. 2003). At present, we have no data on heritability of male traits, male dominance and female choice, and their consequences for offspring fitness. The third possibility is that females solicit sneaking by preferred males. Sneaking is traditionally viewed as alternative male behaviour that further undermines female choice (Taborsky 1998; Jones *et al*. 2001). However, if sneaking males are those preferred by females, their successful fertilizations would tend to augment rather than undermine female choice. Interestingly, our recent findings show that female bitterling may indeed encourage sneaking by particular males (Smith & Reichard, unpublished).

The results of the present study suggest that subordinate males were usually outcompeted by dominant males, either prevented from ejaculating into mussels or in subsequent sperm competition. The capacity to release sperm over the mussel prior to oviposition is a major predictor of relative reproductive success in male bitterling; Reichard *et al*. (2004b) found a strong positive correlation between the proportion of pre-oviposition ejaculations by males and the proportion of fathered offspring. Furthermore, no variation in the number of spermatozoa in ejaculates of individual bitterling has been observed (Candolin & Reynolds 2002). Our original experimental design included measurements of sperm traits, but logistic constraints prevented its completion. Nevertheless, in a separate study with different males, no difference in sperm quality traits and sperm longevity was detected between territorial and sneaker males (M. Reichard, unpublished data), indicating that sperm competition in bitterling may represent a fair raffle (Parker 1998). Thus, the fact that dominant males prevented successful ejaculations from their rivals appears to be the most plausible explanation for the overriding effect of intrasexual competition on the strength of sexual selection in European bitterling.

Our study demonstrates that inter- and intrasexual components of sexual selection may not always operate in concert, and that these conflicts can only be detected through behavioural studies combined with molecular measures of reproductive success. However, their relative importance and their effects on trait expression inevitably varies with study systems, arising from differences in resource investment, parental care, fertilization mode and other parameters (Avise *et al*. 2002). Studies of other mating systems are needed to formulate a more general conclusion on the dynamics of the relationship between pre- and postcopulatory female choice and male–male competition.

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