

Sexual conflict inhibits female mate choice for major histocompatibility complex dissimilarity in Chinook salmon

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In many species females prefer major histocompatibility complex (MHC) dissimilar mates, which may improve offspring resistance to pathogens. However, sexual conflict may interfere with female preference when males attempt to mate with all females, regardless of compatibility. Here we used semi-natural spawning channels to examine how mating behaviour and genetic similarity at the MHC class II peptide binding region affected parentage patterns in Chinook salmon (*Oncorhynchus tshawytscha*). We found that females directed aggression at more MHC-similar males than expected by chance, providing a possible mechanism of female MHC choice in salmon. Males also directed aggression towards MHC-similar females, which was consistent with males harassing unreceptive mates. Males' aggression was positively correlated with their reproductive success, and it appeared to overcome female aversion to mating with MHC-similar males, as females who were the target of high levels of male aggression had lower than expected MHC divergence in their offspring. Indeed, offspring MHC divergence was highest when the sex ratio was female-biased and male harassment was likely to be less intense. These data suggest that male harassment can reduce female effectiveness in selecting MHC-compatible mates, and sexual conflict can thus have an indirect cost to females.

Keywords: salmonids; major histocompatibility complex; mate choice; sexual selection; coercive mating

1. INTRODUCTION

Sexes often face different selective pressures because of unequal parental investment. Females typically have high parental investment and are selective when choosing mates, whereas males typically have low parental investment and are less selective (Trivers 1972). As a consequence of this differential investment in parental effort, there are typically more reproductively active males than females at a given time and thus the operational sex ratio (OSR) is male-biased (Emlen & Oring 1977). This biased sex ratio is associated with stronger sexual selection on males than on females, and can lead to intense competition among males for mates (Clutton-Brock & Parker 1992; Kvarnemo & Ahnesjö 1996). As a consequence of intense competition for mates, and because the reproductive interests of the sexes often differ, sexual conflict may occur, which can ultimately result in the expression of characteristics that are favoured in one sex but decrease the fitness of the other sex (Arnqvist & Rowe 2005).

Harassment is a common form of sexual conflict in which males harass females who resist mating with them to increase their likelihood of mating (Smuts & Smuts 1993; Clutton-Brock & Parker 1995). For example, in fallow deer (*Dama dama*), males often persist in chasing

and attempting to mount females that initially resist their courtship (Clutton-Brock *et al.* 1988). In guppies (*Poecilia reticulata*), males may attempt to copulate by thrusting their gonopodium towards an unreceptive female's genital pore, with females experiencing male harassment as often as once a minute (Magurran & Nowak 1991; Magurran & Seghers 1994b). Male harassment thus generates direct costs for females such as wounds that are incurred during harassment, greater exposure to predators or interference with foraging (Magurran & Seghers 1994a; Rowe 1994; Blanckenhorn *et al.* 2002). Furthermore, females may incur additional costs when males are successful at circumventing female mate preferences. Mate preferences often allow females to obtain either high quality or compatible genes from a mate, with indirect benefits that include improved offspring fecundity, survival and immune response (review in Neff & Pitcher 2005). Thus, male harassment could represent a significant indirect cost for females via reduced genetic quality of their offspring.

The genes of the major histocompatibility complex (MHC) encode proteins that serve an important role in the immune response of vertebrates and are an important component of offspring genetic quality. MHC proteins (class I and II) work by binding fragments of self and foreign peptides and then presenting these peptides on the cell surface to T cells, which initiate an immune response (Janeway *et al.* 1999; Bernatchez & Landry 2003). Class I MHC proteins are expressed on all nucleated somatic cells, and generally recognize

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intracellular pathogens such as viruses. Class II MHC proteins are expressed on antigen-presenting immune cells that include B cells and macrophages, which primarily recognize extracellular pathogens such as parasites and some bacteria (Janeway *et al.* 1999). The amino acid sequence of the MHC molecule, particularly at the peptide-binding region, determines which antigens can be bound, and hence which pathogens can be recognized (Brown *et al.* 1988). Thus, diversity at the MHC locus should allow an individual to respond to a greater range of pathogens (Doherty & Zinkernagel 1975), albeit in species that express multiple MHC loci, an intermediate number of MHC alleles may provide superior pathogen-resistance by lowering the number of T cells that are eliminated by self-reactivity (Nowak *et al.* 1992; Woelfing *et al.* 2009).

There has been considerable research on mate choice and MHC diversity, with evidence that mate choice optimizes MHC variation in the offspring of mammals, birds and fishes (e.g. Reusch *et al.* 2001; Roberts & Gosling 2003; for reviews see Penn 2002; Bernatchez & Landry 2003). Among fishes, the role of MHC genotypes during mating has been best characterized in the stickleback and salmonid families. Both sticklebacks and salmonids have been shown to rapidly detect and respond to olfactory cues of conspecific MHC genotypes (Olsén *et al.* 1998; Milinski *et al.* 2005), providing a mechanism facilitating MHC-mediated mate preferences. Three-spined sticklebacks (*Gasterosteus aculeatus*) are characterized by gene duplication at the MHC class II and individuals with an intermediate number of alleles show greater resistance to multiple pathogens and higher lifetime reproductive success than individuals who have either a high or a low number of MHC alleles (Wegner *et al.* 2003; Kurtz *et al.* 2004; Kalbe *et al.* 2009). Female sticklebacks consequently appear to select mates such that their offspring have the optimal number of MHC alleles (Reusch *et al.* 2001; Milinski *et al.* 2005). Conversely, salmonids typically express a single MHC class II locus (Shum *et al.* 2001), and MHC heterozygosity has been associated with resistance to infectious haematopoietic necrosis virus (*Novirhabdovirus*) in Chinook salmon (Arkush *et al.* 2002), and resistance to bacterial kidney disease caused by *Renibacterium salmoninarum* in Atlantic salmon (*Salmo salar*) (Turner *et al.* 2007). Mate choice in salmonids is consequently predicted to maximize offspring MHC heterozygosity. Indeed, not only high offspring heterozygosity, but high amino acid divergence between the two MHC alleles may be favoured by mate choice, as MHC proteins with similar peptide-binding regions may be more limited in the diversity of pathogens that can be recognized than proteins with less similar peptide-binding regions (Wakeland *et al.* 1990). For example, studies of both Atlantic salmon and Chinook salmon have shown that female mate choice increases the amino acid divergence at the MHC for her offspring relative to the expectations during random mating (Landry *et al.* 2001; Neff *et al.* 2008). Alternatively, in brown trout (*Salmo trutta*), females have been shown to prefer mates with intermediate MHC amino acid divergence, likely because males with high MHC dissimilarity may be less adapted to the local pathogen community (Forsberg *et al.* 2007). Thus, mate choice for genetic

benefits from MHC diversity can be complex, yet there remain few studies in fishes and other animals that examine the behavioural mechanisms that females use to select mates and how males respond to female preferences.

Many aspects of the breeding system of salmonids are well characterized. Salmon are typically anadromous and thus spend most of their life in the ocean before returning to fresh water to breed. The sex ratio of returning salmon is highly variable, and may have a significant bias towards either males or females, with the more common sex outnumbering the rarer sex by more than two to one (Beacham 1984; Fleming 1998). During spawning, females construct and defend nests where they deposit their eggs, whereas males provide only sperm and no parental care (Quinn 2005). Male reproductive success is thus limited primarily by access to breeding females, so males compete intensely with one another to monopolize access to nesting females (Fleming & Gross 1994; Quinn & Foote 1994). Males suffer an opportunity cost by guarding females, as they forsake spawning opportunities with other females and may face selective pressure to minimize the time spent in mate guarding. In contrast, females are not typically limited by access to males and may instead attempt to mate with the highest quality males by, for example, directing aggression towards non-preferred males or delaying spawning when attended by a non-preferred male (Foote 1989; Fleming *et al.* 1997; Berejikian *et al.* 2000). Thus, there is potential for sexual conflict in salmon, as males may increase their reproductive success by harassing unreceptive females that delay spawning.

Here we combine detailed observations of spawning behaviour with genetic analysis of mating patterns in Chinook salmon. We predicted that intrasexual aggression would correlate with reproductive success, as dominant females may obtain preferred nest sites, and dominant males may monopolize access to spawning females. We also examined how intersexual aggression related to MHC genotypes, as we predicted that female aggression towards MHC similar males may be the mechanism that leads to the higher observed MHC amino acid divergence in a female's offspring than expected by chance (Neff *et al.* 2008). We likewise examined the role of MHC genotype in male aggression towards females. In addition, as in Forsberg *et al.* (2007), we examined the possibility that Chinook salmon prefer mates of intermediate MHC dissimilarity by assessing the variance in MHC divergence among mates. Finally, we combined the data of this study with that of a previous study (Neff *et al.* 2008) to examine the effect of OSR and hence the intensity of sexual selection on offspring MHC divergence. We predicted that a male-biased sex ratio would lead to less favourable MHC genotypes in the offspring as more males would be able to circumvent female preferences, as has been inferred in sticklebacks (Eizaguirre *et al.* 2009).

2. MATERIAL AND METHODS

(a) *Experimental fish and set-up*

Experiments were conducted using Chinook salmon at Yellow Island Aquaculture Ltd (YIAL), Quadra Island, British Columbia, Canada. The YIAL population was founded with gametes from the Robertson Creek hatchery

on Vancouver Island, and has been maintained since 1986. MHC diversity in the YIAL population is comparable to other populations of Chinook salmon, in which three to six alleles have been identified within a population (Miller & Withler 1996; Kim *et al.* 1999; Docker & Heath 2002; Pitcher & Neff 2006). YIAL has eliminated the male sex chromosome through the use of hormonal sex-reversal in female XX salmon. Homogametic (XX) males grow to a similar size and mass as heterogametic (XY) males and have similar circulating levels of testosterone and 17 β -oestradiol (Heath *et al.* 2002). XX males also display the same range of spawning behaviours used by XY males and have similar courtship success when in direct competition with XY males in the spawning channels used in the current study (table S1 in the electronic supplementary material).

From 6 to 14 October 2005, mature fish were collected from the YIAL saltwater cages and anaesthetized with buffered MS222 so they could be weighed and a small portion of the adipose fin could be removed and preserved in ethanol for genetic analyses. Each fish was tagged with an individually numbered 3.2 cm diameter Floy tag inserted through the dorsal musculature. While anaesthetized, the left side of each fish was photographed with a digital camera (Fuji Finepix 4900) next to a size standard. These images were used to determine morphological measures comprising total length, height measured at the dorsal fin and height at the caudal peduncle. These three measurements and mass were later collapsed into a single index using principle components analysis (the first axis had positive loadings on all four variables and captured more than 81% of the variation for both males and females).

The fish were then transferred to two freshwater spawning channels. Each channel received 30 fish in approximately a 2:1 male to female sex ratio (19:11 and 18:12). Mature females were all 4 years old, while males included 10 3-year-old fish in each channel and either eight or nine 4-year-old fish. Each channel was 15 \times 3.5 m in size and had a water depth of approximately 1 m. Water flow was provided by pipes at the head of each channel that supplied approximately 80 l min⁻¹ of fresh water and 240 l min⁻¹ of re-circulated water that was collected by pipes at the foot of the channel. The gravel composition was similar to wild streams in the area and measured approximately 3–6 cm in diameter. The channels were located outside, and thus were exposed to natural photoperiod. The channels were surrounded on all sides by netting to exclude predators.

Behaviour was observed continuously during daylight for the duration of the spawning season (11 October to 8 November) by two observers stationed in 5 m high towers overlooking the channels. Whenever an aggressive act was observed (defined as a bite or rapid charge by one fish towards another), the observers recorded the identity of the aggressor and the recipient. The fish were allowed to spawn without interference, and were removed from the channel when they died.

On 30 March 2006 we used seine nets to collect 445 fry from one channel and 190 fry from the other, which were euthanized with an overdose of MS222 and frozen for later genetic analyses.

(b) *Microsatellite and major histocompatibility complex genotypes*

Mating events regularly occurred after dark and were thus not observed, so we used genetic parentage to directly

measure reproductive success for each fish. DNA was extracted from parents and offspring, and genetic variation was then evaluated using seven microsatellite loci (for details see Neff *et al.* 2008). Parental allocation was based on maximum likelihood procedures implemented in the program Cervus (v. 3.0, Marshall *et al.* 1998; Kalinowski *et al.* 2007). We were not always able to obtain genotypes at all seven loci and elected to include only offspring for which we had genotypes at three or more loci (exclusion probability more than 75%). Individual reproductive success was calculated using the sum of offspring assigned to either a parental pair ($n = 500$ offspring) or a single parent ($n = 52$ offspring) at 80 per cent or higher confidence (i.e. a total of 552 offspring were successfully assigned to at least one parent). Analysis of mating patterns used only offspring for which the parental pair was assigned.

Genotypes were determined for a 294 bp portion of the MHC class IIB gene (Docker & Heath 2002) using single strand conformational polymorphism (Amersham Biosciences) to identify unique banding patterns. Each unique pattern was then cloned from two individuals using a Promega pGEM-T easy vector kit and sequenced. Up to four clones were sequenced per individual.

(c) *Mating analysis*

We first examined the phenotypic characteristics associated with reproductive success, which was calculated for each adult as the proportion of the total number of offspring whose parents were identified from that channel. In females, the relationship between reproductive success and body size was assessed using a non-parametric Spearman correlation. Because males of multiple ages were present, the effect of body size on male reproductive success was analysed using an analysis of covariance that included age (3, 4 years) as a fixed factor and body size as a covariate. In each sex the frequencies of aggression towards both males and females were calculated as the proportion of the acts of that type recorded in the channel; these frequencies were compared with reproductive success using non-parametric correlations. Frequencies of aggression were also compared with age (males only) and body size as described for the comparisons of reproductive success.

Longevity was calculated for each fish as the number of days between when they were released in the channel and when they died. The rates of both intersexual and intrasexual aggression towards each fish were then calculated as the total number of observed aggressive acts of that type divided by the longevity of the fish. We used non-parametric correlations for each sex to compare rates of aggression to longevity. The rates allowed us to examine the cost of being targeted by aggression while controlling for the greater number of observations of fish with high longevity. Longevity was also compared with age (males only) and body size as described for the comparisons of reproductive success.

We used a Monte Carlo simulation to examine whether aggression between potential mates was non-random with respect to MHC genotype (Manly 1997). Our null hypothesis was that intersexual aggression was random with respect to MHC amino acid divergence. The simulation for female aggression towards males analysed 121 aggressive interactions in which both a male and a female were identified. For each aggressive act, the simulation randomly assigned a male from the channel as the target, and then calculated the expected amino acid divergence for the pair

(see Landry *et al.* 2001 for calculation of amino acid divergence). The average and variance for the 121 values were calculated, and the routine was repeated for a total of 5000 simulations. Male aggression towards females was analysed similarly using simulations based on 32 aggressive interactions in which both a male and a female were identified. For each sex, the values for average MHC amino acid divergence and variance in MHC amino acid divergence were sorted and the observed values from the actual data were compared with the distributions to determine p -values.

Following Neff *et al.* (2008), we used a Monte Carlo simulation to determine if non-random mating in Chinook salmon produced offspring with greater MHC amino acid divergence or lower variance in MHC amino acid divergence than expected by chance. Briefly, a simulation randomly assigned mates to females based on their observed number of mates (as calculated from the parentage analysis). The expected MHC amino acid divergence in the offspring was then calculated based on the assigned males and their skew in paternity (see Neff *et al.* 2008). The simulation was repeated 5000 times and the distribution was used to calculate p -values. The data from the current study were then combined with those presented in Neff *et al.* (2008) to examine the effect of sex ratio on MHC-mating patterns. The study of Neff *et al.* (2008) examined mating patterns in groups of 36 Chinook salmon held in the same freshwater channels at YIAL, although it did not include behavioural observations of spawning fish. Overall, six channels were examined across 2 years, comprising three channels at a 2:1 sex ratio, one channel at a 1:1 sex ratio, and two channels at a 1:2 sex ratio. First, we compared offspring MHC divergence at a 2:1 sex ratio using an analysis of variance (ANOVA) that included year as a fixed factor and channel as a nested factor within year. This analysis was used to ensure that there was no difference in the mean amino acid divergence between years in equivalent sex ratio treatments. Next, for all females from both years we calculated a relative MHC divergence index by subtracting the offspring amino acid divergence expected under random mating (i.e. mean divergence of the female with each male in her channel) from the female's actual offspring amino acid divergence as determined from the parentage analysis. We analysed the relative MHC divergence index using an ANOVA that included sex ratio as a fixed factor (1:2, 1:1 and 2:1) and channel as a random nested factor within sex ratio. Channel was included to control for any differences in the mating conditions experienced by individual fish among the six channels used across the 2 years. Statistical analyses were performed using JMP (v. 4.0.4, SAS Institute Inc.).

3. RESULTS

There was considerable variation in aggression among both males and females (figure 1). During the spawning season we observed females performing 194 aggressive acts towards other females and 121 acts towards males. Female body size was not correlated with either the rate of aggression towards other females ($r_s = 0.13$, $n = 23$, $p = 0.55$) or towards males ($r_s = 0.25$, $n = 23$, $p = 0.24$). The rate of female aggression towards other females was highly correlated with the rate of aggression towards males ($r_s = 0.71$, $n = 23$, $p < 0.001$). Males were observed performing 439 aggressive acts towards other males and 32 acts towards females. The rate of

aggression towards other males was not related to either male age ($F_{1,34} = 2.71$, $p = 0.11$) or body size ($F_{1,34} = 0.72$, $p = 0.40$). The rate of aggression towards females was similarly unrelated to either male age ($F_{1,34} = 0.50$, $p = 0.48$) or body size ($F_{1,34} = 0.01$, $p = 0.91$). The rate of male aggression towards other males was highly correlated with the rate of aggression towards females ($r_s = 0.55$, $n = 37$, $p < 0.001$).

Female longevity in the spawning channels ranged from 16 to 37 days (mean = 25 days), and was not correlated with body size ($r_s = -0.03$, $n = 23$, $p = 0.89$). The rate of intrasexual aggression towards a female was negatively correlated with her longevity ($r_s = -0.50$, $n = 23$, $p = 0.015$). In contrast, the rate of male aggression towards a female was positively correlated with her longevity ($r_s = 0.47$, $n = 23$, $p = 0.025$). Male longevity in the spawning channels ranged from 13 to 39 days (mean = 28 days), and was not related to either age ($F_{1,34} = 0.01$, $p = 0.91$) or body size ($F_{1,34} = 1.07$, $p = 0.31$). The rate of intrasexual aggression towards a male was not correlated with his longevity ($r_s = 0.06$, $n = 37$, $p = 0.71$). However, the rate of female aggression towards a male was negatively correlated with his longevity ($r_s = -0.45$, $n = 37$, $p = 0.005$).

There was no relationship between a female's reproductive success and body size ($r_s = 0.26$, $n = 23$, $p = 0.23$). Female reproductive success was also not correlated with her relative aggression towards other females ($r_s = 0.21$, $n = 23$, $p = 0.34$) or towards males ($r_s = 0.35$, $n = 23$, $p = 0.10$). A female's number of mates was not correlated with her relative aggression towards males ($r_s = 0.26$, $n = 23$, $p = 0.23$). Male reproductive success was not related to either male age ($F_{1,34} = 0.28$, $p = 0.60$) or body size ($F_{1,34} = 0.01$, $p = 0.92$). Male reproductive success was not correlated with his relative aggression towards other males ($r_s = 0.23$, $n = 37$, $p = 0.17$), but was positively correlated with his relative aggression towards females ($r_s = 0.47$, $n = 37$, $p = 0.003$). A male's number of mates was also positively correlated with his relative aggression towards females ($r_s = 0.49$, $n = 37$, $p = 0.002$).

The sequence analysis revealed four MHC alleles, each of which encoded a different amino acid sequence. These MHC alleles comprised the three most common alleles identified in the YIAL population during the 2004 mating season (Neff *et al.* 2008), as well as a novel allele that differed from a common allele by a single amino acid (figure S1 in the electronic supplementary material). Behavioural observations showed that patterns of aggression during mating were non-random with respect to MHC genotypes. Females directed aggression towards males with whom they would produce offspring with significantly lower MHC dissimilarity than expected with a random male ($p = 0.023$; figure 2a), and males also directed aggression towards females with whom they would produce offspring with significantly lower MHC dissimilarity than expected with a random female ($p = 0.037$; figure 2b). Examining female aggression towards males, three acts were followed by aggression by that male towards the female, two acts involved simultaneous reciprocal aggression, 11 acts were preceded by aggression by that male towards the female, and 105 acts were directed at males who were not aggressive towards the female. Examining male aggression towards

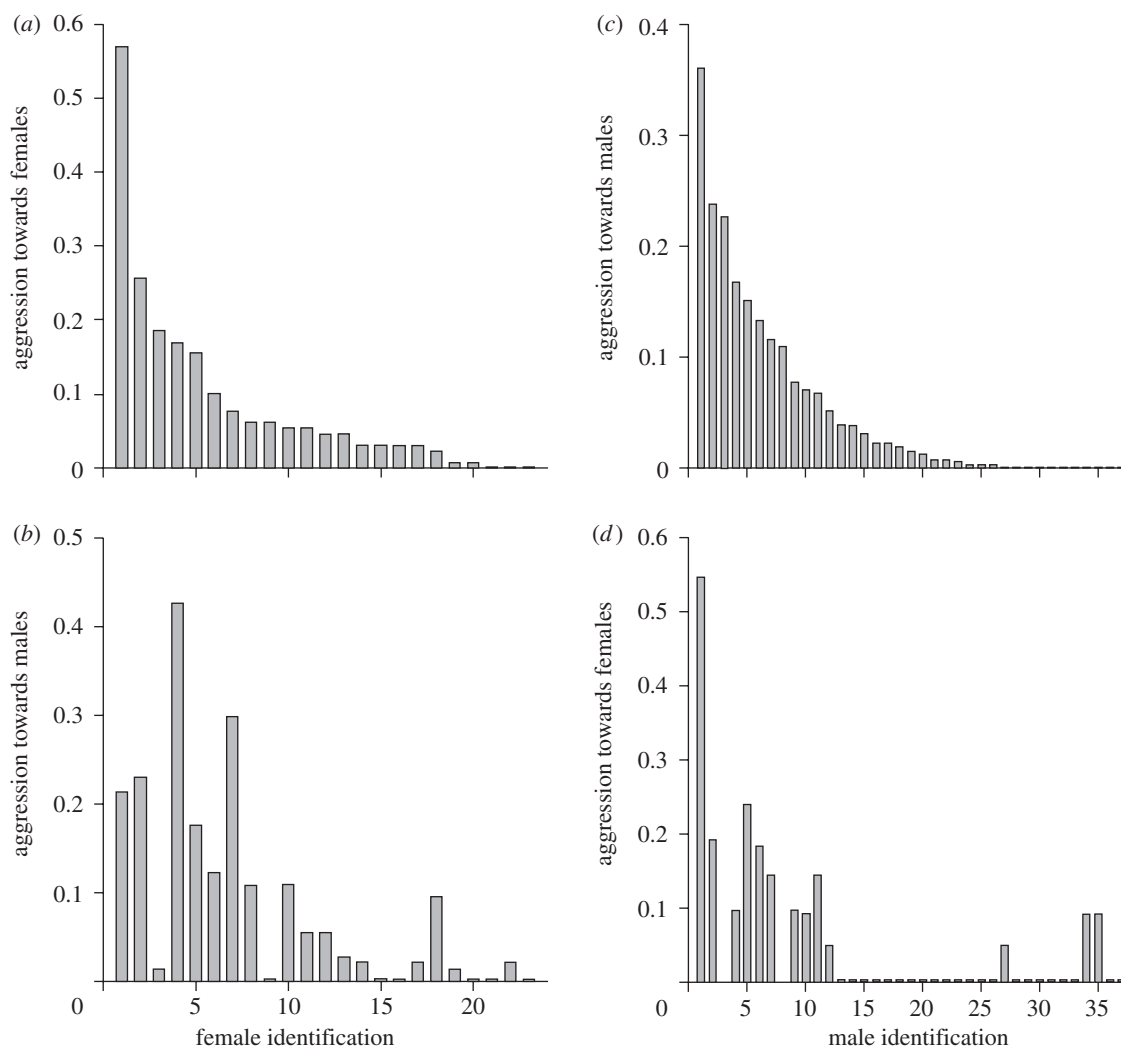


Figure 1. Summary of intersexual and intrasexual aggression in Chinook salmon (*Oncorhynchus tshawytscha*). Data represent the frequency of aggression by females towards (a) other females and (b) males, and the frequency of aggression by males towards (c) other males and (d) females. Male and female identification is based on rank order for intrasexual aggression (highest to lowest). The same identification numbers are used for females in panels (a) and (b), and for males in panels (c) and (d). Aggression for each individual was calculated within each channel as the proportion of the acts of that type; thus the values within each panel sum to 2 ($1 \times$ number of channels).

females, seven acts were followed by aggression by that female towards the male, two acts involved simultaneous reciprocal aggression, one act was preceded by aggression by that female towards the male, and 22 acts were directed at females who were not aggressive towards the male. In males, frequency of aggression towards females was positively correlated with female aggression towards that male ($r_s = 0.45$, $n = 37$, $p = 0.005$). In contrast, female aggression towards males was unrelated to the frequency of male aggression towards that female ($r_s = 0.12$, $n = 23$, $p = 0.58$). The rate at which females directed aggression towards a male was negatively correlated with his relative MHC divergence index ($r_s = -0.36$, $n = 35$, $p = 0.035$; figure 3a), and the rate at which males directed aggression towards a female was negatively correlated with her relative MHC divergence index ($r_s = -0.47$, $n = 23$, $p = 0.024$; figure 3b).

The Monte Carlo simulation of mating revealed that females did not achieve higher MHC divergence in their offspring than expected under random mating ($p = 0.80$). These data were not statistically different than the 2:1 male:female sex ratio trial done in 2004 (year

effect: $F_{1,32} = 0.37$, $p = 0.55$). However, when data from all three sex ratios were combined, we found that female-biased sex ratios led to higher offspring MHC divergence than expected by chance, while male-biased sex ratios led to significantly lower MHC divergence ($F_{2,81} = 11.9$, $p = 0.006$; figure 4). No significant difference in this pattern was detected among channel replicates at each sex ratio ($F_{3,81} = 0.21$, $p = 0.89$), indicating that this trend was consistent among groups of fish in different channels.

We found no evidence that Chinook salmon preferred mates with intermediate MHC dissimilarity. The Monte Carlo simulation of mating revealed that females did not have lower variance in the MHC dissimilarity of the alleles in their offspring than expected under random mating ($p = 0.36$). Female aggression was not directed towards males with whom they would produce offspring with higher variance in MHC dissimilarity than expected with a random male ($p = 0.39$). Male aggression was also not directed towards females with whom they would produce offspring with higher variance in MHC dissimilarity than expected with a random female ($p = 0.14$).

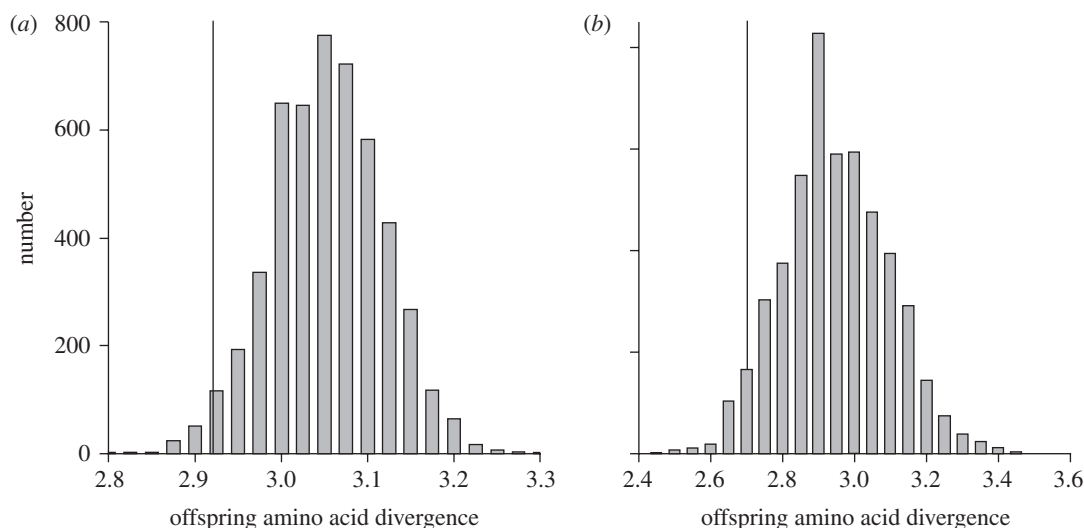


Figure 2. Summary of the Monte Carlo simulations for tests of non-random intersexual aggression in Chinook salmon (*Oncorhynchus tshawytscha*). Data represent the distributions of 5000 simulations for the expected value under random aggression for (a) female aggression towards males and (b) male aggression towards females. The vertical line in each panel denotes the observed value.

4. DISCUSSION

Here we have shown that intersexual aggression is a strong determinant of mating patterns in Chinook salmon. Males with high rates of aggression towards females had higher reproductive success than less aggressive males, whereas in females the rate of aggression towards males was unrelated to reproductive success. However, female aggression was directed at more MHC-similar males than expected by chance, which is consistent with females avoiding MHC-similar mates. Male aggression was also directed at more MHC-similar females than expected by chance, yet was preceded by female aggression towards that male less than 10 per cent of the time. As such, this pattern cannot be explained solely by reciprocal aggression. Instead, this pattern is consistent with males harassing unreceptive females to obtain spawning opportunities. Indeed, male aggression seemed to overcome female aversion to mating with MHC-similar males, as females who were the target of high levels of male aggression had lower than expected MHC divergence in their offspring. Our combined analysis of the 2004 and 2005 mating trials revealed that female Chinook salmon achieved significantly higher offspring MHC divergence only when the sex ratio was female-biased.

Female preference for MHC compatibility may be expressed through a number of mechanisms. Our findings show that in Chinook salmon, females preferentially direct aggression towards MHC-similar males. As Chinook salmon females, and not males, defend spawning territories, female aggression may discourage a male from courting a female and serve as a mechanism facilitating MHC compatibility. In three-spined sticklebacks, males instead guard the nests and females are free to choose among males based on MHC genotype without directed aggression (Reusch *et al.* 2001; Milinski *et al.* 2005). In humans, females find the scent of males with dissimilar MHC genotypes more attractive (Wedekind & Furi 1997), and this preference also translates into mate choice, as fewer couples share MHC alleles than expected by chance (Ober *et al.* 1997). In mice (*Mus musculus*) and

savannah sparrows (*Passerculus sandwichensis*), females can use extra-pair matings to find MHC-dissimilar males and produce offspring of high genetic quality (Potts *et al.* 1991; Freeman-Gallant *et al.* 2003; Roberts & Gosling 2003). Thus, females may be free to choose among males, use aggression to drive away incompatible males, or use extra-pair matings to obtain MHC-compatible males.

Males, on the other hand, may use aggression towards females to coerce individuals who may otherwise be reluctant to mate. Evidence that males harass females to obtain mating opportunities has been documented in a number of species, which include mammals, birds, insects and fish (Smuts & Smuts 1993; Magurran & Seghers 1994b; Clutton-Brock & Parker 1995). Harassment of unreceptive females may be an effective male reproductive tactic because males have much lower parental investment in offspring than females, and as such typically benefit more from mating with a large number of females than from selecting only the highest quality mating opportunities (Bateman 1948; Trivers 1972). In our study, sexual coercion appeared to be a successful male tactic for obtaining spawning opportunities, as male aggression towards females was positively correlated with that male's number of mates and consequently his reproductive success. Sexual coercion thus allowed males to mate with females of both high and low MHC dissimilarity. Although the fitness value of mating with MHC-similar females is unknown, male fitness is almost certain to increase as a consequence of producing more offspring than if they mated only with MHC-dissimilar females. Male aggression towards females has similarly been interpreted as sexual coercion in Coho salmon (*Oncorhynchus kisutch*) (Watters 2005), which suggests that sexual conflict may be a common characteristic of salmonid breeding systems.

Male sexual coercion may be costly to females. First, females may suffer direct costs such as injury from male harassment. In our study, high intrasexual aggression towards females was associated with reduced longevity,

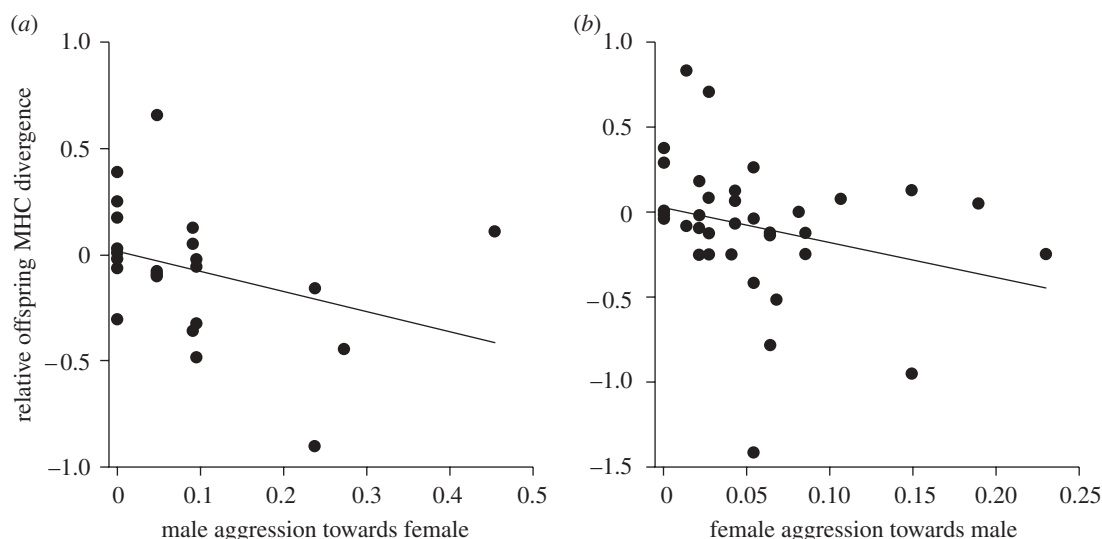


Figure 3. Relationship between intersexual aggression towards an individual and offspring MHC divergence in Chinook salmon (*Oncorhynchus tshawytscha*). Data represent (a) male aggression towards a female and the relative MHC divergence of her offspring and (b) female aggression towards a male and the relative MHC divergence of his offspring. Relative MHC divergence was calculated as the observed amino acid deviation for that individual's offspring minus the expectation for that individual under random mating. Rates of aggression were standardized across channels by expressing aggression as the proportion of aggressive acts in each channel. The line presents the result of a linear regression.

albeit the rate of male aggression towards a female was actually positively correlated with her longevity. It is possible that the costs of male harassment may have been masked by males predominantly directing aggression towards females in good condition. In many other mating systems, male aggression has been linked to female injury and reduced longevity (e.g. Le Boeuf & Mesnick 1991; Blanckenhorn *et al.* 2002). Second, females may incur indirect costs from harassment through circumvention of their mate choice. We found that male harassment of females counteracted female preference for MHC-dissimilar mates. In the rose bitterling (*Rhodeus ocellatus*), it has been shown that females who mate with preferred males have offspring with higher survival than females who mate with non-preferred males (Casalini *et al.* 2009). However, when multiple males competed to spawn with females, male dominance, and not female preference, was the primary determinant of mating patterns and often led females to mate with non-preferred males (Casalini *et al.* 2009). Thus, sexual coercion by males can affect female fitness directly through reduced longevity or indirectly through reduced offspring genetic quality.

The OSR appears to have important consequences on male harassment and female opportunities to select mates. Male harassment of females is typically higher in male-biased sex ratios as there is higher competition among males for mating opportunities (Emlen & Oring 1977; Sih & Krupa 1995; Jirotkul 1999; Smith & Sargent 2006). Although our study did not compare male behaviour at multiple sex ratios, we were able to examine the outcome of mating based on sex ratio. Combining our data here with that from a previous study (Neff *et al.* 2008), we found that females achieved significantly higher offspring MHC divergence at a female-biased sex ratio than at a male-biased sex ratio. This result is consistent with decreased harassment by males at a lower male sex ratio, which could release females

from the negative effects of harassment on their ability to select mates. Male-biased sex ratios have also been shown to reduce the ability of large males to monopolize access to spawning females, which increases the opportunity for small males to sneak access to spawning females (Fleming & Gross 1994). Sneaking behaviour can reduce the ability of females to select MHC-compatible mates as has been shown in three-spined sticklebacks, particularly at high male densities (Eizaguirre *et al.* 2009). Indeed, although we did not observe any obvious sneaking behaviour, it may also be contributing to the MHC differences that we observed among sex ratios. Overall, in species where males can interfere with female mate choice, the expression of female-mating preferences may depend on the OSR or other determinants of male opportunity to circumvent female preferences.

It is not clear if mating preferences in salmonids commonly favour intermediate offspring MHC diversity. In brown trout, lower than expected variance in MHC divergence between mating pairs has been interpreted as evidence that mate choice favours intermediate MHC divergence (Forsberg *et al.* 2007). In the current study, however, we found no evidence that female Chinook salmon produced offspring with lower than expected variance in MHC amino acid divergence, as would be expected if females preferred mates with intermediate MHC divergence. Aggression towards potential mates was likewise random with respect to the expected variance in MHC amino acid divergence in the offspring. Our results also contrast those from three-spined sticklebacks, in which mate choice for an intermediate number of offspring MHC alleles is well established (e.g. Reusch *et al.* 2001; Milinski *et al.* 2005). This discrepancy probably relates to the fact that sticklebacks express multiple MHC class II loci, whereas salmonids typically express a single MHC class II locus. Negative selection of T cells in the thymus has been estimated to lead to

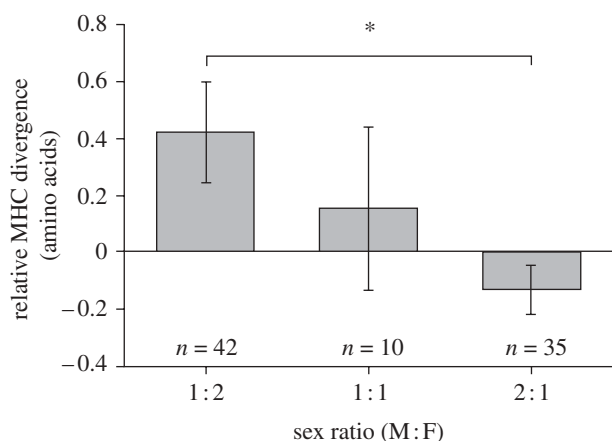


Figure 4. Relative offspring MHC divergence observed among parental sex ratios in female Chinook salmon (*Oncorhynchus tshawytscha*). Means are presented with ± 1 s.e. The sample sizes present the number of females in each sex ratio that were analysed. Significant differences between sex ratios are indicated with an asterisk.

maximum pathogen resistance when 3–25 MHC alleles are expressed (Woelfing *et al.* 2009), so even when salmonids express two highly divergent MHC alleles it is unlikely that negative selection of T cells would lead to reduced pathogen resistance. In brown trout, females may instead prefer intermediate MHC diversity to avoid mating with highly divergent, non-local males that may not be adapted to the local pathogen community (Forsberg *et al.* 2007). Understanding population genetic structure and local adaptation to pathogen communities will no doubt help to clarify apparent differences in mating preferences for MHC divergence among salmonid species.

Our study is among the first to show that male harassment has negative effects on females' ability to select MHC compatible mates, and indicates that female preferences for MHC-dissimilar mates can be insufficient to infer actual mating patterns. Thus, although mate choice has been implicated as a major mechanism maintaining polymorphism at MHC loci (Bernatchez & Landry 2003), sexual coercion can counteract mate choice and may actually reduce such polymorphism. Additional behavioural and genetic studies of mating at sex ratios that include female-biased ratios will help to more fully detail the influence of sexual coercion on mating patterns at the MHC. Furthermore, although sexual coercion no doubt increases male fitness by expanding the range of females available as mates, the magnitude of this benefit remains to be determined. Assessing the relative value of offspring with low MHC amino acid dissimilarity and the costs of harassment for males, including injuries from reciprocal female aggression, will help to clarify the circumstances in which males are likely to benefit from sexual coercion. Finally, it is unclear at what point during breeding MHC genotype is assessed, and thus whether or not males can alter their courtship behaviour to manipulate or mask their MHC genotype when courting MHC-similar females. Understanding the role of sexual conflict in shaping mating patterns related to the MHC thus remains a rich area for further study.

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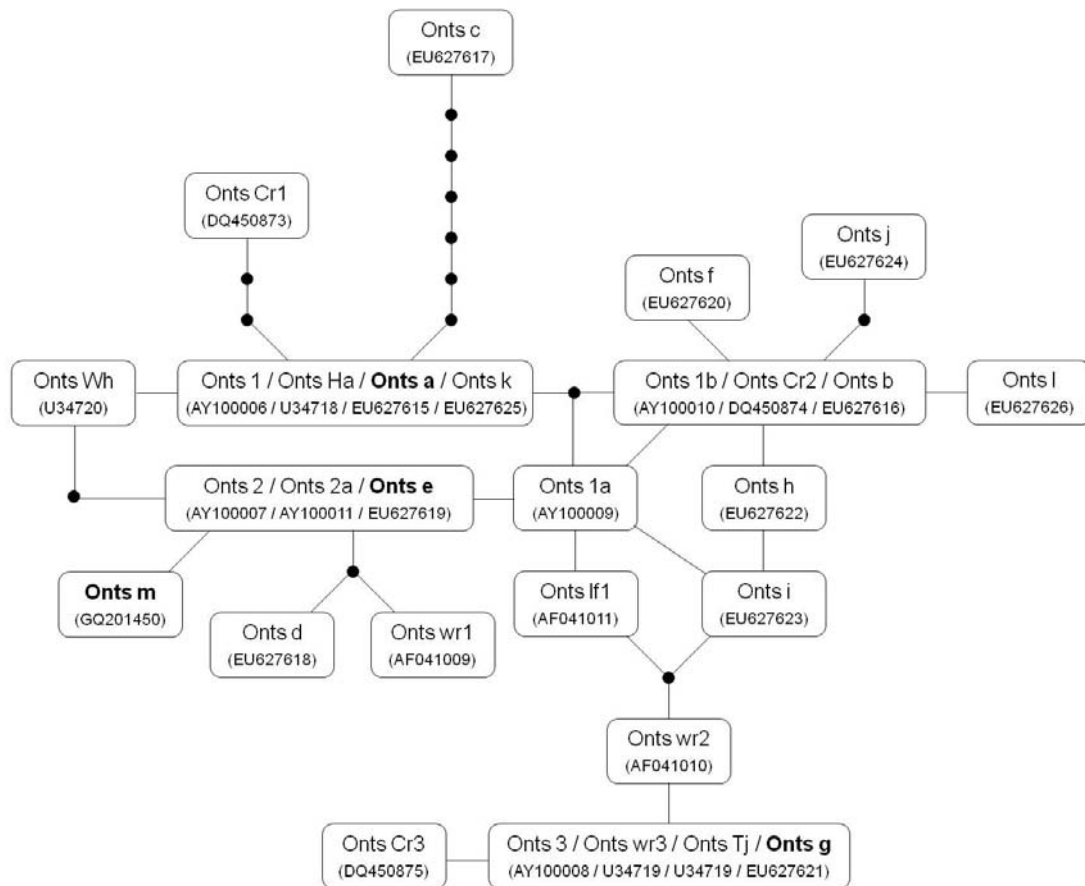
1 **Table S1.** Comparison of spawning behaviour in homogametic (XX) and heterogametic
2 (XY) male Chinook salmon (*Oncorhynchus tshawytscha*). Observations were conducted
3 using a separate freshwater spawning channel at YIAL in which 10 three-year-old males (3
4 XY; 7 XX), 9 four-year-old males (3 XY; 6 XX), and 11 four-year-old females were
5 allowed to spawn. Heterogametic (XY) males were produced by fertilizing eggs from
6 YIAL females with the milt of males from the Quinsam River Hatchery on Vancouver
7 Island, BC, Canada. Both XX and XY males were reared from hatching until maturity at
8 YIAL. Results show the mean \pm SE for each measurement as well as the outcome of t-tests
9 that compared XX and XY males.

	XX males	XY males	t	P
Longevity (days)	28.8 \pm 1.2	30.7 \pm 1.0	0.99	0.34
Aggressive acts directed at males (#)	10.0 \pm 4.6	3.3 \pm 1.6	0.96	0.35
Aggressive acts directed at females (#)	0.6 \pm 0.5	0.0 \pm 0.0	0.89	0.38
Aggressive acts received from males (#)	6.5 \pm 1.1	4.0 \pm 1.6	1.27	0.22
Aggressive acts received from females (#)	1.3 \pm 0.4	1.0 \pm 0.5	0.41	0.68
Quivering towards females (#)	2.2 \pm 0.7	0.5 \pm 0.3	1.52	0.15

10

1 **Figure S1.** Summary of the 19 amino acid sequences identified at the peptide binding
2 region of the MHC class IIB locus in Chinook salmon (*Oncorhynchus tshawytscha*). The
3 diagram depicts the presumed evolutionary relationship between the alleles where each dot
4 denotes one amino acid substitution. The relationship among the MHC alleles was
5 determined using statistical parsimony, as implemented in the program TCS (Clement *et*
6 *al.* 2000). Sequences were originally reported in Miller & Withler (1996; Onts Ha, Tj;
7 Wh), Kim *et al.* (1999; Onts lf1, wr1, wr2, wr3), Docker & Heath (2002; Onts 1, 1a, 1b, 2,
8 2a, 3), Pitcher & Neff (2006; Onts Cr1, Cr2, Cr3), and Neff *et al.* (2008; Onts a, b, c, d, e,
9 f, g, h, i, j, k, l). Alleles that did not differ in protein sequence are separated with a slash
10 and Genbank accession numbers are provided below all alleles. Bold text is used to
11 indicate alleles that were found in the present study.

12



13 **References**

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