ARTICLE

Behavioural and genetic analyses of mate choice and reproductive success in two Chinook salmon populations

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Abstract: Sexual selection is recognized as an important evolutionary force in salmon. However, relatively little is known about variation in sexual selection pressures across salmon populations or the potential role of natural selection as a driver of adaptive mating patterns. Here, we examine mating behaviour and correlates of reproductive success in Chinook salmon (*Oncorhynchus tshawytscha*) from the Quinsam and Little Qualicum rivers in British Columbia, Canada — two populations for which we have previously found evidence of natural selection operating on major histocompatibility complex (MHC) genes. In both populations, males courted females and exhibited dominance behaviour towards other males, and the frequency of each behaviour was positively associated with reproductive success. Males were more aggressive towards females with whom they would produce offspring of low or high MHC class II diversity, and the offspring of males from the Quinsam River exhibited higher diversity at the MHC class I than expected. We discuss our results in relation to local natural selection pressures on the MHC and the potential for MHC-dependent mate choice.

Résumé : La sélection sexuelle est reconnue comme étant un important moteur d'évolution chez le saumon. On en sait cependant assez peu sur la variation des pressions de sélection sexuelle d'une population de saumons à l'autre ou sur le rôle éventuel de la sélection naturelle comme déterminant des modes d'accouplement adaptatifs. Nous examinons le comportement d'accouplement et les corrélats du succès de reproduction chez le saumon quinnat (*Oncorhynchus tshawytscha*) des rivières Quinsam et Little Qualicum, en Colombie-Britannique (Canada), deux populations pour lesquelles nous avons déjà démontré la présence d'une sélection naturelle s'opérant sur des gènes du complexe majeur d'histocompatibilité (MHC). Dans les deux populations, les mâles courtisent les femelles et présentent un comportement de dominance envers les autres mâles, et la fréquence de ces comportements est positivement associée au succès de reproduction. Les mâles sont plus agressifs envers les femelles avec lesquelles ils vont produire une descendance de diversité faible ou forte au niveau de la classe II du MHC et la descendance des mâles de la rivière Quinsam présente une plus grande diversité que prévu au niveau de la classe I du MHC. Nous discutons de nos résultats en ce qui concerne les pressions de sélection naturelle locales sur le MHC et la possibilité d'une sélection de partenaire dépendant du MHC. [Traduit par la Rédaction]

Introduction

In salmonid fishes, sexual selection has led to much of the diversity we see in mating behaviour and secondary sexual characteristics (de Gaudemar 1998). Salmonids have also evolved in the face of spatially and temporally variable natural selection pressures (e.g., Fleming and Gross 1989; Hamon and Foote 2005; Dionne et al. 2007; Evans et al. 2010a, 2010b , 2010c; see Fraser et al. 2011 for a review), which may influence adaptive mating decisions across years or populations (see de Gaudemar 1998; Hamon and Foote 2005). However, relatively little is known about the interplay between natural and sexual selection or the congruency of mating patterns across populations of salmon (de Gaudemar 1998; but see Quinn 1999).

Studies of major histocompatibility complex (MHC) genes provide us with a unique opportunity to examine links between natural and sexual selection processes. MHC molecules represent the interface between the vertebrate immune system and parasites infecting the host, both recognizing and presenting parasite-derived peptides to T-cells. Thus, MHC genes are the likely targets of parasite-driven natural selection pressures (Spurgin and Richardson 2010). MHC genes may also be the subjects of sexual selection pressures if parents are able to improve the "genetic

quality" of offspring though MHC-dependent mate choice (Neff and Pitcher 2005; e.g., Penn and Potts 1999; Freeman-Gallant et al. 2003; Brouwer et al. 2010). Indeed, it has been suggested that MHC-dependent mate choice should be disassortative, as offspring bearing a diverse complement of MHC alleles may be better able to mount immune responses against parasites than their less diverse counterparts (Penn and Potts 1999). Mate choice may also target a "good" MHC allele when a population is subjected to a prevalent pathogen (Neff and Pitcher 2005) or intermediate levels of diversity when, for example, individuals face trade-offs between the benefits of maximizing offspring MHC genetic diversity and the potential costs of outbreeding depression (Forsberg et al. 2007; also see Milinski et al. 2005; Eizaguirre et al. 2009; Kalbe et al. 2009). Thus, while MHC-dependent mating preferences are generally considered adaptive, these preferences may vary among populations and are likely to depend on the nature of parasite-driven natural selection operating within a population.

In this study, we examine MHC-dependent mate choice and behavioural and morphological correlates of reproductive success in the Chinook salmon (*Oncorhynchus tshawytscha*). Our study focuses on spawning populations found in the Quinsam and Little Qualicum rivers on Vancouver Island, British Columbia. Previous research on MHC class I and II population genetics and parasite

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Published at www.nrcresearchpress.com/cjfas on 18 December 2012.

infections of Vancouver Island Chinook salmon suggests that parasite-driven natural selection is operating on these genes (Evans and Neff 2009; Evans et al. 2010a). Specifically, in the Quinsam River and Big Qualicum River (a river located 15 km from the Little Qualicum River), offspring with MHC class I dissimilar parents exhibit higher survivorship compared with the offspring of parents bearing more similar alleles (Evans et al. 2010b). In contrast, the offspring of MHC class II similar parents exhibit higher survival than those of MHC dissimilar parents, at least in the Quinsam population (Evans et al. 2010b). MHC class I molecules generally recognize antigens derived from intracellular parasites such as viruses, whereas class II molecules generally recognize antigens derived from extracellular parasites such as macroparasites or bacteria (Klein et al. 2007). Therefore, the differences in natural selection pressures detected at the two MHC loci and across populations could be related to the differing parasite communities recognized by class I and II molecules.

We collected detailed observations of spawning interactions between male and female salmon and through genetic parentage analysis, examined the reproductive success of males and females within each river. For each population, we also examined the influence of MHC allele similarity between males and females on mating behaviour and reproductive success, in addition to potential relationships between morphological and behavioural traits and reproductive success. We predicted that if sexual selection reinforces natural selection, mating behaviour should promote diversity at the MHC class I but possibly select for reduced or intermediate levels of diversity at the class II locus (see Evans et al. 2010b).

Methods

The Quinsam and Little Qualicum rivers occur approximately 120 km apart on the east coast of Vancouver Island, British Columbia. We obtained Chinook salmon for use in our study from hatchery brood stock at the Fisheries and Oceans Canada Little Qualicum River hatchery, Qualicum Beach, British Columbia, on 3 October 2006, and the Fisheries and Oceans Canada Quinsam River hatchery, Campbell River, British Columbia, on 20 October 2006. At each hatchery, brood stock was collected by staff while the salmon undertook freshwater migration to their spawning grounds. Following collection, the salmon were held in large holding channels located adjacent to the river. Because the hatcheries clip the adipose fin of juveniles upon release, we assumed that all individuals used in our study were of wild origin, as they all bore intact adipose fins.

All individuals were sedated with buffered MS-222 (tricaine methanesulfonate), weighed, individually marked with a 3.2 cm diameter Floy disc tag attached through the musculature located directly anterior to the dorsal fin, and photographed with a digital camera (Canon Powershot S1 IS). All photographs included a size standard and were subsequently used to measure the postorbital hypural body length, dorsal height, caudal peduncle width, and snout length of each individual (see Fleming and Gross 1994). A small portion of the adipose fin was also collected from each individual and stored in 95% ethanol for genetic analysis.

Following tagging, individuals were placed into an oxygenated recovery tank and allowed to regain consciousness prior to introduction into the spawning channels. For the Little Qualicum River population, a total of 21 males and 11 females were introduced into a seminatural spawning channel at the Little Qualicum hatchery. For the Quinsam River population, a total of 17 males and 9 females were introduced into a seminatural spawning channel at the Oyster River Enhancement Society hatchery, which is approximately 22 km south of the Quinsam River hatchery. We selected "ripe" females (i.e., females whose eggs were ready to be laid) by applying light pressure to the abdomen and ensuring that eggs were freely released from the ovipositor. The number of males and females included in each spawning channel were selected to mirror the approximately 2:1 male-to-female sex ratio that is typical of the natural populations (Fisheries and Oceans Canada Big Qualicum and Quinsam hatcheries, unpublished data). Both channels measured approximately 25 m in length and 10 m in width and contained natural gravel bottoms as spawning substrate. The water depth within each channel ranged between 1 and 1.5 m. Water was diverted from the main stem of the Little Qualicum River and Oyster River into each spawning channel. Thus, water conditions reflected those found in each of the rivers. Each channel was contained within a fenced enclosure and netted from above to exclude predators.

Behavioural observations

A 1 m × 1 m grid system was erected above each spawning channel, and with reference to the grid, two observers positioned at opposite ends of the spawning channel, and hidden behind camouflaged blinds, recorded all mating interactions occurring during daylight hours and until all spawning females had died. This technique has been used previously to assess behavioural interaction rates in both the coho (Oncorhynchus kisutch) and Chinook salmon (Fleming and Gross 1994; Garner et al. 2010). In total, we observed spawning behaviour for Little Qualicum and Quinsam fish over 20 days and 15 days, respectively. However, for the Quinsam River, 5 days of observation were finally excluded from analyses as a result of heavy rainfall and silt occluding visibility.

We recorded incidences of intersexual and intrasexual aggression and male courting (see Supplemental Materials Table S1[1] for definitions of behaviour). Behavioural frequency was assessed using counts. For male attendance, a male was "counted" as attending to a female once until that behaviour was interrupted. If a male returned to the female to continue attending, we would count that as a separate event. On most days, males were counted as attending to a female only once, and thus the counts represent conservative estimates of effort. A measure of "dominance", derived from intrasexual aggression behaviour, was determined for each adult using David's score (see Gammell et al. 2003). For behavioural rates exhibited by individuals, all counts were standardized by the total number of days an individual was observed. For behavioural interactions between male–female pairs, we standardized counts by the number of days a putative pair could be observed together.

In some cases, an individual's identifying tag was lost, so we were able to record only behavioural interactions for that individual up to the date of tag loss. In the Little Qualicum spawning channel, two females and seven males lost their tags immediately following introduction into the spawning channel and thus were excluded from any analyses examining genetic or phenotypic correlates of behavioural interactions. For all other fish, tags were retained for at least 10 days following introduction into the spawning channel.

Offspring parentage analysis

Between 4 and 11 December 2006, Chinook salmon offspring were collected from each spawning channel using hydraulic sampling (i.e., by pumping water through a probe into the spawning substrate). Previous studies have shown that a large proportion of spawning activity in salmon occurs at night (Berejikian et al. 2000). Because we were only able to observe spawning activity during daylight hours, it is possible that many of the redds and individual nests within redds remained undocumented. There-

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[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2012-0415.

fore, we sampled for embryos in a systematic (every 1 m × 1 m grid location) manner throughout each spawning channel. In total, we collected 208 developing embryos from the Quinsam spawning channel and 230 embryos from the Little Qualicum spawning channel, which were stored in 95% ethanol for future parentage analysis. While these sample sizes are relatively modest, they reflect the difficulty associated with sampling salmon embryos from spawning substrate (see Berejikian et al. 2011). Also, because hydraulic sampling depends on water pressure to loosen embryos from gravel, this method is likely best able to purge nests located at shallower depths. Thus, it is possible that this technique could be biased towards females who have superimposed their nests over those of other females, or more generally, towards nests built at shallower depths.

We used the DNA Wizard Extraction kit (Promega Corp., Madison, Wisconsin, USA) to isolate DNA from the adipose tissues of parents and the embryos. We genotyped all parents, all embryos collected from the Little Qualicum population, and 202 of 208 embryos collected from the Quinsam population at eight microsatellite loci (Ots107, Ots104, OtsG311, OtsG249, Omy325, OtsG83B, OtsG68, OtsG432; Table S2[1]). Each locus was amplified using polymerase chain reaction (PCR). The PCR amplification conditions consisted of an initial DNA denaturation step for 60 s at 94 °C, followed by 35 cycles of 60 s at 94 °C, 60 s at a locus-specific annealing temperature (see Table S2[1]), extension for 60 s at 72 °C, followed by a final extension step at 72 °C for 90 s. Reactions consisted of 1 μL DNA template, 1 μL 10× reaction buffer, 2.5 mmol·L^{-1} MgCl$_2$, 0.2 mmol·L^{-1} each dNTP, 2.0 μmol·L^{-1} of each primer, 0.5 U Taq polymerase (GIBCO BRL, Gaithersburg, Maryland), and ddH2O up to a 10 μL reaction volume. Each forward primer was tagged with a fluorescent dye, which enabled the PCR products to be compared against size standards on the Li-Cor 4300 DNA analyzer (Li-Cor Biosciences, Lincoln, Nebraska). For each locus, we calculated the number of alleles and the expected (H_E) and observed (H_O) heterozygosity found in the Quinsam and Little Qualicum parents in Genepop version 3.4 (Raymond and Rousset 1995; see Table S2[1]).

We used the program CERVUS version 3.0 (Kalinowski et al. 2007) to assign offspring to the most likely parental pair. Parentage analysis in CERVUS consists of an initial simulation that evaluates the power of the genetic markers used to assign offspring to their parents. The simulations for both Quinsam and Little Qualicum parents indicated that our suite of eight loci is capable of assigning 100% of the offspring to a parental pair with an 8% misassignment rate. We applied an additional restriction on our parentage assignments: the requirement for a positive LOD score for each parental pair – offspring match. A positive LOD score indicates that the candidate parental pair is more likely to be the true parental pair than not. Following the application of this restriction, a total of 148 and 158 offspring were retained for our examinations of genetic and phenotypic correlates of reproductive success for the Quinsam and Little Qualicum populations, respectively.

Reproductive success was calculated for each male or female as the proportion of offspring assigned to the individual out of the total number of offspring assigned via parentage analysis in each population. To determine the reproductive success of a parental pair, we calculated "relative reproductive success", which is defined as the total number of offspring identified to the parental pair / (the total number of offspring identified to the male + the total number of offspring identified to the female – the total number of offspring identified to the parental pair). The measure corrects for any bias related to differences in the number of offspring sampled from each male and female.

MHC genetic analysis

In teleost fishes, MHC classes I and II are unlinked and thus may be examined independently for their roles in adaptive evolution (Bingulac-Popovic et al. 1997). We genotyped all parents at the MHC class I-A1 and MHC class II-B1 loci using primer sets described in Grimholt et al. (1993) and Hordvik et al. (1993), respectively. These loci are expressed and associated with the peptide-binding region of MHC molecules. MHC loci were amplified using PCR following the protocols outlined in Miller and Withler (1997) and Docker and Heath (2002). The MHC class I primers amplify either 222 or 228 base pairs (bp), whereas the class II primers amplify 213 bp. MHC PCR products were visualized using SSCP (single strand conformation polymorphism) electrophoresis on the Amersham-Biosciences SSCP system (GE Healthcare Life Sciences, Baie d'Urfe, Canada). Samples that showed a unique SSCP pattern (genotype) were cloned using the Promega pGEM T-easy vector kit (Promega Corp.) and had between four and eight clones sequenced to determine the allelic composition of the genotype. Amplification products were sequenced by Genome Québec (McGill University, Montréal, Canada), and sequences were aligned using the Clustal W algorithm (Thompson et al. 1994) in MEGA version 4 (http://www.megasoftware.net/; Tamura et al. 2007). MHC class I sequences were aligned to sequences associated with the human leukocyte antigen (HLA; i.e., human MHC) A1 locus (Bjorkman et al. 1987), and MHC class II sequences were aligned to sequences associated with the HLA DRB-1 locus (Brown et al. 1993). These alignments were used to identify the nucleotides that encode the putative peptide-binding region (PBR) — the nucleotides directly involved in binding to pathogens — in Chinook salmon based on the known PBR of HLA molecules. Many of these sites show evidence of positive selection, suggesting that they play an integral role in pathogen resistance (Evans et al. 2010a).

We calculated nucleotide dissimilarity (D_{xy}) at the MHC class I and II loci between each male and female by summing the number of nucleotide differences between their MHC alleles (i.e., parental alleles = A, a, B, b; $D_{xy} = D_{AB} + D_{Ab} + D_{aB} + D_{ab}$; also see Landry et al. 2001). A relatively large number of nucleotide differences between parental MHC alleles will result in a high D_{xy} value, whereas few nucleotide differences will result in a low D_{xy} value. D_{xy} between a parental pair is a useful way of estimating how "diverse" the MHC alleles found in their offspring would be (i.e., higher dissimilarity between parents should result in the offspring bearing a more diverse complement of MHC alleles; but see Yeates et al. 2009) and the degree of disassortative (or assortative) mating occurring in a population. We examined D_{xy} across the entire MHC allele sequence and separately for nucleotides associated with the PBR. However, the results from both measurements were similar; thus, we report the results based on the more conservative PBR-only measurement.

Statistical analysis

Morphological measurements were collapsed into a single index termed "body size" using principle components analysis (PCA) on correlations. Two-way ANOVA was used to compare the masses of males and females in each of the populations. We used independent samples t tests (assuming unequal sample sizes and variances between populations) to examine differences in the MHC D_{xy} of putative parental pairs between the two populations. To examine variation in overall rates of intrasexual and intersexual interactions between the two populations, we used a one-way ANOVA that incorporated the amount of time (hours) we observed fish each day multiplied by the number of males or females observed in each population as a weighting factor.

The estimates of reproductive success followed an approximate Poisson distribution. Thus, we square-root (reproductive success + 0.5)-transformed our data as suggested by Zar (1999). Given that there were a large number of potential explanatory variables associated with male and female reproductive success, we initially used stepwise linear regression to determine the "best" model for describing variation in reproductive success. The "best" model was selected according to the minimum Akaike information cri-

terion (AIC). Generalized linear models were then used to examine the relationship between male or female reproductive success and the factors selected by stepwise regression. For the male stepwise regression model, male intersexual aggression, male courting, dominance, body size, population, and the interactions between population and the other factors were initially included as potential explanatory variables (Table S3[1]). The female model included female intersexual aggression, dominance, body size, population, and the interactions between population and the other factors as potential explanatory variables (Table S3[1]).

We used a similar model reduction approach to examine the relationship between MHC D_{xy} and a parental pair's relative reproductive success. In the initial stepwise linear regression model MHC class I and class II D_{xy}, the quadratic predictors (class I D_{xy})² and (class II D_{xy})², population, and the interactions between population and the MHC D_{xy} variables were included as potential explanatory variables. Male and female ID were also included as fixed factors to account for multiple observations per individual (Table S3[1]). In this model a positive or negative association between a simple MHC D_{xy} factor and relative reproductive success of the parental pairs would suggest that matings occur more frequently between individuals bearing more MHC dissimilar or similar alleles, respectively. A significant relationship between an MHC quadratic factor (i.e., D_{xy}^2) and relative reproductive success would suggest that matings occur more or less frequently between parental pairs bearing intermediate rather than low or high levels of MHC dissimilarity.

We used a model similar to the relative reproductive success model to examine potential relationships between MHC dissimilarity and male courting, male intersexual aggression, and female intersexual aggression behaviour (Table S3[1]).

To further investigate relationships between MHC dissimilarity and the mating patterns of males and females identified through genetic parentage analysis, we conducted permutation tests in Excel 2007 (Good 2000). Permutation tests are distribution-free tests and thus are robust to the assumptions of parametric statistical approaches. For each population, the mean nucleotide diversity expected in the offspring of males or females, at the MHC class I or class II locus (mean D_{xyPop}), was calculated. An example of how this calculation was performed is shown for the offspring of males:

$$\text{mean } D_{xyPop} = \frac{1}{N_M} \sum_{ij} \left[\frac{OFF_{ij}}{OFF_i} \times D_{xy(ij)} \right]$$

where OFF_{ij} is the total number of offspring assigned to male i and female j, OFF_i is the total number of offspring assigned to male i, $D_{xy(ij)}$ is the number of nucleotide differences between the MHC alleles of male i and female j, and N_M is the total number of males in the population with offspring assigned to them. An analogous calculation was conducted for females.

We then compared the observed mean D_{xyPop} with that expected under patterns of random mating. For instance, in the permutation test examining male mating patterns, the mean D_{xyPop} observed for the class I or class II was compared with a distribution of mean D_{xyPop} values generated by randomizing the number of offspring a male sired with each female across all females. The randomization was repeated for 2000 iterations to generate the distribution of mean D_{xyPop} values expected under random mating. When the observed mean D_{xyPop} value fell significantly ($P < 0.05$) outside of the permutation distribution, this was taken as evidence of a relationship between the MHC and male mating patterns (see Good 2000). We used a similar permutation approach to examine whether males and females select mates with respect to variance in MHC D_{xy} (i.e., using average variance in population D_{xy} values rather than absolute D_{xy} values; see Fors-

berg et al. 2007). This simulation allowed us to examine whether patterns of mate choice by males or females were highly congruent (low variance) or variable (high variance) for offspring MHC diversity.

All statistical analyses, excluding the permutation tests, were performed in JMP (version 9, SAS institute), and a significance value of 0.05 was used for all tests. Means are reported ±1 SD.

Results

Male and female reproductive success

Male and female reproductive success was highly variable in both populations. For Little Qualicum fish, the reproductive success (represented as a proportion of all offspring genotyped) of females ranged between 0 and 0.50, and we assigned offspring to six females through parentage analysis (see Supplemental Materials, Fig. S1a[1]). For males, reproductive success ranged between 0 and 0.47, and we assigned offspring to 15 males through parentage analysis (Fig. S1b[1]). There was variation in the number of males siring offspring with each female; females one through six (Fig. S1a[1]) had offspring sired by three, six, three, two, four, and one male(s), respectively.

For Quinsam females, we assigned offspring to six females via parentage analysis, and reproductive success ranged between 0 and 0.56 (Fig. S1c[1]), whereas for Quinsam males, reproductive success ranged between 0 and 0.46, and a total of nine males had offspring assigned to them through parentage analysis (Fig. S1d[1]). Similar to what we found in Little Qualicum, the number of males siring offspring with each female varied in the Quinsam population; females one through six (Fig. S1c[1]) had offspring sired by four, six, one, two, one, and one male(s), respectively.

Behavioural and morphological variation

The Little Qualicum females examined in this study averaged 7.8 ± 1.4 kg and the males averaged 5.3 ± 2.2 kg. Quinsam females averaged 11.2 ± 2.8 kg and males averaged 9.4 ± 2.2 kg. Thus, the Quinsam females and males were both significantly heavier than their counterparts in the Little Qualicum population (two-way ANOVA: $F_{[3,58]} = 21.1$, $P < 0.001$; population: $F_{[1]} = 42.1$, $P < 0.001$), and females were significantly heavier than the males in each population ($F_{[1]} = 9.1$, $P = 0.004$).

In the Little Qualicum population, the PCA analysis of body size explained 74% and 67% of the variation among males and females, respectively (PCA loadings; males: mass = 0.49, length = 0.48, dorsal height = 0.48, caudal width = 0.38, snout length = 0.38; females: mass = 0.42, length = 0.50, dorsal height = 0.46, caudal width = 0.52, snout length = 0.30). In the Quinsam population, the first PCA axis explained 64% and 62% of the variation in male and female body size, respectively (PCA loadings; males: mass = 0.52, length = 0.46, dorsal height = 0.49, caudal width = 0.48, snout length = 0.21; females: mass = 0.54, length = 0.54, dorsal height = 0.27, caudal width = 0.51, snout length = −0.26).

Over the 20 days of observation in the Little Qualicum population and the 10 days of observation in the Quinsam population, we observed 704 (out of a total of 1816 acts) and 345 (out of a total of 404 acts) behavioural interactions for which the identity of both individuals involved could be determined, respectively (see Table 1). The lower proportion of behavioural acts identified to an individual in the Little Qualicum population compared with the Quinsam population was related to the number of identifying tags lost in the former, and this influenced the reproductive success estimates that could be used in our analysis of behavioural predictors of reproductive success. For the Little Qualicum population, we ultimately obtained behaviour estimates from 10 (of 15) males and 5 (of 6) females for whom we also assigned offspring through parentage analysis. But overall, the two populations exhibited similar standardized rates of intersexual aggression by

Table 1. Summary of the total number of spawning behaviours observed over 20 and 10 days, respectively, in the Little Qualicum River and Quinsam River Chinook salmon (*Oncorhynchus tshawytscha*) populations.

	Little Qualicum	Quinsam	F	df	P
Intrasexual aggressive acts by females	458	107	3.39	1,28	0.076
Intersexual aggressive acts by females	164	10	0.72	1,28	0.402
Intrasexual (dominance) aggressive acts by males	649	99	0.01	1,28	0.933
Intersexual aggressive acts by males	185	7	4.14	1,28	0.051
Courting acts by males	360	181	2.66	1,28	0.114
Total	1816	404			

Note: See supplementary Table S1[1] for a definition of each spawning behaviour. No population-level differences were significant following Bonferroni correction (*P* value/5). One-way ANOVA, incorporating time observed per day × number of individuals observed as a weighting factor, was used to examine differences in behavioural rates between the populations. *F* statistics and numerator and denominator degrees of freedom (df) for the model are shown.

males and females, male courting, and male and female dominance behaviour (Table 1).

According to the stepwise regression model, female dominance, intersexual aggression, and body size were not significantly associated with female reproductive success (Table S3[1]). However, male reproductive success was positively associated with male courting and male dominance (Table 2; Figs. S2a, S2b[1]), but negatively associated with male body size (Table 2; Fig. S2c[1]).

MHC genetic variation

We identified a total of 18 MHC class I alleles and six MHC class II alleles in the Little Qualicum adults. A total of 15 MHC class I and four MHC class II alleles were identified in the Quinsam adults (see Supplemental Materials, Table S4 for allele frequencies and Fig. S3 for amino acid sequences[1]). Mean MHC class I D_{xy} between all putative parental pairs in each of the populations did not differ significantly (Quinsam: 19.4 ± 6.7, Little Qualicum: 20.3 ± 6.1, $t_{[302]} = -1.21$, $P = 0.22$). However, Little Qualicum parents exhibited significantly higher MHC class II D_{xy} (11.6 ± 8.5) compared with Quinsam parents (6.7 ± 4.9; $t_{[375]} = -7.14$, $P < 0.001$).

According to the stepwise linear regression models, none of the MHC genetic dissimilarity factors were significantly associated with a parental pair's relative reproductive success or male courting or female intersexual aggression behaviour. However, the quadratic MHC class II D_{xy} factor (class II D_{xy}^2) was significantly associated with male intersexual aggression, with the highest levels of male aggression directed towards MHC class II similar and dissimilar females (Table 2; Fig. S4[1]). Given that little male intersexual aggression was observed in the Quinsam population, the relationship between the MHC class II and intersexual aggression was largely driven by observations from the Little Qualicum population (Fig. S4[1]).

For Little Qualicum females, the permutation tests revealed that predicted mean offspring MHC class I and class II D_{xy} were not significantly different than expected under random mating (mean MHC class I $D_{xyPop} = 19.8$, $P = 0.72$; mean MHC class II $D_{xyPop} = 11.6$, $P = 0.71$; Figs. 1a, 1b). Similar results were found for the offspring of Little Qualicum males (mean MHC class I $D_{xyPop} = 21.5$, $P = 0.18$; mean MHC class II $D_{xyPop} = 12.3$, $P = 0.35$; Figs. 1c, 1d). For Quinsam females, the predicted MHC class I and class II D_{xy} found in offspring was also not significantly different than expected from random mating (mean MHC class I $D_{xyPop} = 21.2$, $P = 0.21$; mean MHC class II $D_{xyPop} = 6.7$, $P = 0.35$; Figs. 1e, 1f). However, the Quinsam males mated more frequently with females with whom they would produce offspring bearing highly dissimilar MHC class I

Table 2. Summary of generalized linear models examining (*i*) morphological and behavioural traits associated with male reproductive success and (*ii*) the influence of MHC class II nucleotide dissimilarity (D_{xy}) on the frequency of male-driven intersexual aggression in Chinook salmon (*Oncorhynchus tshawytscha*) from the Quinsam and Little Qualicum populations.

Predictor	R^2	B	df	F	P
(*i*) Male reproductive success					
Model	0.44		3,29	8.73	**<0.001**
Dominance		0.05	1	6.51	**0.017**
Body size		−0.49	1	6.67	**0.016**
Courting		175.52	1	14.04	**<0.001**
(*ii*) Male intersexual aggression					
Model	0.17		4,296	16.54	**<0.001**
Male ID		−0.0215	1	32.49	**0.009**
Class II D_{xy}		0.0001	1	0.02	0.888
Class II D_{xy}^2		0.0002	1	4.16	**0.042**

Note: The models were reduced to the "best" model through stepwise linear regression using the minimum Akaike information criterion, as indicated in the Methods. The effect (*B*) of each factor on the dependent, and the significance of the relationship between each of the factors and the dependent, examined using the *F* statistic, are shown. Degrees of freedom (df) are indicated for each factor within the model, and numerator and denominator df are indicated for the model. Significant relationships are indicated with bold *P* values.

(mean $D_{xyPop} = 23.9$, $P = 0.04$; Fig. 1g) but not MHC class II (mean $D_{xyPop} = 5.9$, $P = 0.31$; Fig. 1h) alleles.

Similar to our results examining patterns of nonrandom mating for MHC dissimilarity, most of the permutation tests suggested that mating patterns for variance in offspring MHC D_{xy} were not significantly different from random expectations (see Supplemental Materials Fig. S5[1]). However, Quinsam females' offspring had significantly lower variance in MHC class II D_{xy} than expected from random expectations (mean variance in $D_{xy} = 4.6$, $P = 0.03$; Fig. S5f[1]).

Discussion

In this study, we examined associations between morphological and behavioural traits and reproductive success in two populations of Chinook salmon. We found that both male courting and dominance were positively associated with reproductive success, supporting what has previously been shown in studies of pink salmon (*Oncorhynchus gorbuscha*; Dickerson et al. 2004) and sockeye salmon (*Oncorhynchus nerka*; Mehranvar et al. 2004). Interestingly, male body size was negatively associated with reproductive success, in contrast with what has been shown in other studies of salmonids (e.g., Quinn and Foote 1994; Fleming and Gross 1994; Ford et al. 2008; Neff et al. 2008; Anderson et al. 2010; see de Gaudemar 1998 for a review). However, our analysis of body size effects on reproductive success controlled for the influence of male dominance and courting behaviour, two traits that tend to co-vary with body size, and thus may reflect the reproductive success of subordinate males using alternative reproductive tactics, such as sneaking to obtain mating opportunities (Mehranvar et al. 2004). For females, we found little evidence that reproductive success was influenced by intersexual aggression or dominance. Similarly, in a study of sockeye salmon, female aggression was not associated with patterns of reproductive success determined through genetic analyses (Mehranvar et al. 2004). In salmonids, it is likely that reproductive success is influenced by factors other than the behavioural tactics examined here. Indeed, male genetic quality, female egg or genetic quality (maternal effects), and the genetic compatibility between males and females have been shown to influence reproductive success (Gage et al. 2004; Yeates et al. 2009; Evans et al. 2010c), though evaluation of such cryptic mating system components is difficult outside of a controlled, lab-based environment. Overall, our results from both

Fig. 1. Summary of the permutation tests of nonrandom mating for MHC nucleotide dissimilarity between male and female Chinook salmon (*Oncorhynchus tshawytscha*). The histogram in each panel shows the predicted distribution of offspring MHC class I or II nucleotide diversity (mean D_{xyPop}) under random mating (i.e., following 2000 randomizations of reproductive success values across males or females). The results of the permutation tests are shown for (*a*) Little Qualicum females at the MHC class I, (*b*) Little Qualicum females at the MHC class II, (*c*) Little Qualicum males at the MHC class I, (*d*) Little Qualicum males at the MHC class II, (*e*) Quinsam females at the MHC class I, (*f*) Quinsam females at the MHC class II, (*g*) Quinsam males at the MHC class I, and (*h*) Quinsam males at the MHC class II. The vertical line in each panel denotes the observed mean D_{xyPop} value.
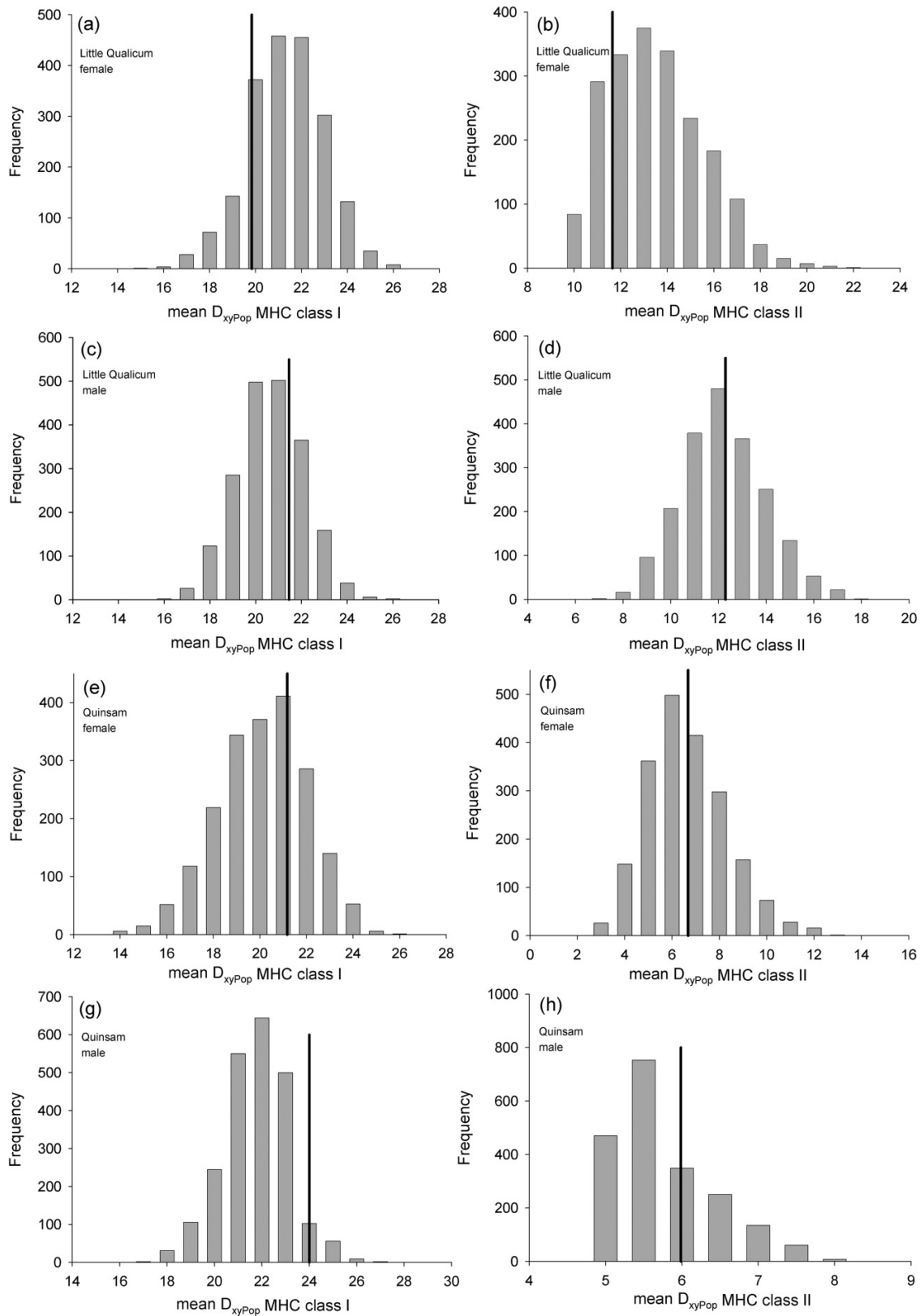
the Little Qualicum and Quinsam populations largely support those of the collective studies of salmonid mating systems, which suggest that male, but not female, mating behaviour is a strong predictor of variation in reproductive success.

Previous research on Chinook salmon from the Qualicum and Quinsam rivers has shown that offspring survival is influenced by MHC class I diversity (Evans et al. 2010b), and we predicted that adaptive MHC-dependent mate choice should evolve in these populations in response to natural selection pressures. Our genetic parentage analyses provided support for this prediction in the Quinsam population where males appeared to mate more frequently with MHC class I dissimilar females, a mating preference that should maximize allele diversity in the males' offspring. We found little evidence that male courting or aggression was influenced by male MHC dissimilarity to females, but it is possible that MHC-dependent mating patterns are also being driven by cryptic tactics that we were unable to observe as part of a study of semi-natural spawning patterns. For instance, males have been shown to increase sperm allocation towards females with preferred MHC genotypes (see Gillingham et al. 2009), and egg fertilization success may depend on MHC allele similarity between mates (Yeates et al. 2009). Results from our parentage analyses also indicate that sperm competition is likely in the populations studied here, where up to six males were observed siring offspring with an individual female. Thus, although we did not identify behavioural preferences for MHC dissimilar mates, the nonrandom mating for MHC dissimilarity by Quinsam males suggests that cryptic MHC-dependent mate choice mechanisms may be operating.

Based on previously observed patterns of offspring survival in the Quinsam population, we predicted that adaptive mate choice should favour matings between males and females bearing intermediate or low levels of MHC class II allele dissimilarity (Evans et al. 2010b). We found some support for this hypothesis; the mean level of MHC class II D_{xy} expected in Quinsam females' offspring, based on parentage assignment, was 6.7 despite the potential for the D_{xy} values at this locus to range up to 13. This observation, in combination with the low predicted variance in offspring MHC class II D_{xy}, suggests that the preferred level of allele dissimilarity between a female and her mate(s) is congruent across females and favours an intermediate level of allele dissimilarity. We also found some evidence that male intersexual aggression is associated with MHC class II dissimilarity, as Little Qualicum males exhibited lower levels of aggression towards females bearing MHC class II alleles of intermediate dissimilarity to their own. This behaviour could be interpreted as a form of mate choice, although it did not appear to influence the predicted allele diversity of males' offspring. Interestingly, intersexual aggression was also the only male behaviour not associated with reproductive success, suggesting that it is not an effective mate choice tactic (also see Mehranvar et al. 2004).

Overall our results indicate that mating behaviour exhibited by Quinsam and Little Qualicum Chinook salmon is largely congruent. Of the different components of behaviour examined, male courting and dominance were associated with increased reproductive success, as was smaller male body size, suggesting that these traits are sexually selected. The MHC class I allele diversity found in males' offspring (predicted from genetic mating patterns) indicated that male-driven sexual selection likely reinforces natural selection, at least in the Quinsam population, where we have previously observed a survival advantage for offspring with MHC class I dissimilar parents. Additionally, genetic patterns of mating by Quinsam females were suggestive of mate choice for intermediate MHC class II diversity in offspring. Thus, female mate choice may also be adaptive, as previous findings have shown that offspring survival is highest for parental pairs exhibiting intermediate or low levels of class II allele similarity (Evans et al. 2010b). Despite finding some evidence for behavioural, genetic, and morphological predictors of reproductive success in our study, it is nevertheless important to note that our estimates

of reproductive success could be biased towards individuals with more readily accessible nests because of the logistical difficulties associated with sampling embryos from spawning substrate. Moreover, our relatively modest sample sizes could limit our power to detect relationships between the examined traits and reproductive success. Thus, our results should be treated with some caution, and indeed, further studies are needed to examine the congruency of these mating patterns across multiple years. Over the long term, it would be particularly useful to follow temporal and spatial covariation in MHC genetic structure, MHC-dependent mating patterns, and the parasite communities (i.e., sources of natural selection) found in each of these populations for further evidence of parasite-driven behavioural and genetic adaptive evolution.

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