

Sperm competition, but not major histocompatibility divergence, drives differential fertilization success between alternative reproductive tactics in Chinook salmon

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Abstract

Post-copulatory sexual selection processes, including sperm competition and cryptic female choice (CFC), can operate based on major histocompatibility (MH) genes. We investigated sperm competition between male alternative reproductive tactics [jack (sneaker) and hooknose (guard)] of Chinook salmon (*Oncorhynchus tshawytscha*). Using a full factorial design, we examined *in vitro* competitive fertilization success of paired jack and hooknose males at three time points after sperm activation (0, 15 and 60 s) to test for male competition, CFC and time effects on male fertilization success. We also examined egg-mediated CFC at two MH genes by examining both the relationship between competitive fertilization success and MH divergence as well as inheritance patterns of MH alleles in resulting offspring. We found that jacks sired more offspring than hooknose males at 0 s post-activation; however, jack fertilization success declined over time post-activation, suggesting a trade-off between sperm speed and longevity. Enhanced fertilization success of jacks (presumably via higher sperm quality) may serve to increase sneaker tactic competitiveness relative to dominant hooknose males. We also found evidence of egg-mediated CFC (i.e. female \times male interaction) influencing competitive fertilization success; however, CFC was not acting on the MH genes as we found no relationship between fertilization success and MH II β_1 or MH I α_1 divergence and we found no deviations from Mendelian inheritance of MH alleles in the offspring. Our study provides insight into evolutionary mechanisms influencing variation in male mating success within alternative reproductive tactics, thus underscoring different strategies that males can adopt to attain success.

Introduction

Sexual selection has long been recognized as a powerful evolutionary process having important consequences for reproductive behaviour, morphology and fitness (Andersson, 1994; Arnqvist & Rowe, 2005; Andersson & Simmons, 2006). Sexual selection processes can include those that operate pre- and post-copulation, where the

latter has often been overlooked until more recently (Birkhead & Pizzari, 2002; Eberhard, 2015; Firman *et al.*, 2017). Post-copulatory sexual selection operates through two potential mechanisms: sperm competition and/or cryptic female choice (CFC). Sperm competition occurs when sperm from multiple males competes for fertilization opportunities (Parker, 1970), whereas CFC occurs when the female influences the outcome of fertilization (Eberhard, 1996). Although the mechanisms for sperm competition vary across taxa, the outcome can typically be predicted based on relative sperm numbers, sperm speed, timing of insemination and timing of female ovulation (Birkhead & Pizzari, 2002; Firman *et al.*, 2017). In contrast, identifying CFC is challenging as it is often less

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evident and can be masked by male-driven components, such as sperm quality (Birkhead, 1998; Birkhead & Pizzari, 2002; Snook, 2005; Firman *et al.*, 2017).

In vertebrates, several studies on sexual selection have explored the possibility that both female mate choice (pre-copulatory) and CFC (post-copulatory) may operate via variation at the major histocompatibility complex (MHC) genes (Ziegler *et al.*, 2005; Firman *et al.*, 2017). MHC genes play a direct role in the immune response, as the genes code for cell-surface receptors that bind self- and non-self-peptides, which are then presented to T cells (Lawlor *et al.*, 1990). There are two functionally distinct MHC molecules, class I and class II, which bind peptides derived from intracellular (often viral) and extracellular (often bacteria) antigens, respectively. In mammals, MHC class I and class II genes are located in close proximity forming a linkage group or 'complex' (Klein, 1986). Conversely, in teleost fishes, the genes encoding the class I and class II genes are unlinked; thus, they are more correctly referred to as major histocompatibility (MH) genes (Stet *et al.*, 2002). The sequences encoding the peptide-binding region (PBR) of the MHC molecule are highly polymorphic; thus, MHC genes have become the candidate genes for numerous studies of sexual selection (Milinski *et al.*, 2005; Ziegler *et al.*, 2005; Kamiya *et al.*, 2014).

Although many studies examine the mechanisms of precopulatory MHC-mediated mate choice (reviewed in Tregenza & Wedell, 2000; Milinski, 2006; Kamiya *et al.*, 2014), there is also evidence that the MHC may also play a role in post-copulatory sexual selection, presumably through egg-sperm interactions (Wedekind *et al.*, 1996; Rüllicke *et al.*, 1998; Yeates *et al.*, 2009; Gasparini *et al.*, 2015; Gessner *et al.*, 2017a). Salmonid species are excellent model systems for the study of post-copulatory sexual selection because fertilization occurs externally, allowing *in vitro* fertilizations under controlled conditions. Furthermore, the structure of the teleost egg consists of an outer envelope (the chorion) and has only one point of entry into the egg (micropyle) (Kamler, 1992). Unlike mammals, only a single sperm is allowed entry into the egg via the micropyle thus driving sperm competition (Kamler, 1992). The micropyle may facilitate CFC because as the sperm moves through the micropyle, the potential exists for the egg to affect fertilization success. The mechanism for such an interaction is not known, but could be mediated by MH allele compatibility, as research in salmonids found that under sperm competition, males with more similar MH genotypes relative to the female outcompeted males with more dissimilar genotypes (Yeates *et al.*, 2009; Gessner *et al.*, 2017a). However, other studies have shown no relationship between MH divergence and competitive fertilization success in salmonids (Skarstein *et al.*, 2005; Lehnert *et al.*, 2017).

In Chinook salmon (*Oncorhynchus tshawytscha*), males compete aggressively for access to spawning females to

increase the probability that their sperm will fertilize the eggs (Berejikian *et al.*, 2001, 2010). Male Chinook salmon exhibit alternative reproductive tactics, where males that mature precociously are referred to as 'jacks', whereas males with later maturation are referred to as 'hooknose' males (Healy, 1991; Heath *et al.*, 1994; Flannery *et al.*, 2013). The larger hooknose males with well-developed secondary sexual characteristics generally achieve dominance in the hierarchy and guard females and thus have a competitive advantage over the smaller jacks (Gross, 1985; Esteve, 2005; Butts *et al.*, 2012a). Therefore, the smaller and cryptic coloured jacks have developed an alternative strategy to obtain fertilizations using a 'sneaking' tactic (Berejikian *et al.*, 2010; Berejikian & Tezak, 2005; Esteve, 2005). During spawning events, jacks emerge from hiding and 'sneak' into position near the female's eggs, allowing jacks, under some conditions, to achieve paternity success comparable to that of hooknose males (Berejikian *et al.*, 2010). Because the quality of available refuges for jacks may not always be favourable for their reproductive success (i.e. reduced effectiveness of sneaking behaviour), selection may lead to increased jack sperm competitiveness to increase fertilization success. Indeed, jack Chinook salmon have faster-swimming sperm than hooknose males (Flannery *et al.*, 2013) and jacks attain greater paternity success when under sperm competition with hooknose males (Young *et al.*, 2013; Lehnert *et al.*, 2017). Although jacks may have a competitive advantage under sperm competition, studies have also identified evidence that CFC may influence success of alternative tactics (Young *et al.*, 2013; Alonzo *et al.*, 2016; Lehnert *et al.*, 2017).

To determine the role of sperm competitiveness and CFC on success of alternative tactics, we investigate the outcome of post-copulatory processes between tactics in Chinook salmon males using a 5 × 5 full factorial *in vitro* fertilization design. Using a full factorial design, we are able to determine the effects of male, female and their interaction on fertilization success. We evaluate *in vitro* competitive fertilization success of paired jack and hooknose males at three time points post-sperm activation (0, 15 and 60 s), where genetic markers are used to determine paternity and quantify male fertilization success. The amount of time required for fertilization to occur under natural spawning events is unknown; however, our time points represent both ecologically relevant time points (0 and 15 s) and a time point representing a scenario of extreme longevity (60 s). We may expect fertilization success of tactics to vary over time post-sperm activation, as sneaking jacks may have evolved faster sperm to allow their sperm to reach the egg quicker and compete directly with hooknose sperm for fertilization. Alternatively, jack sperm may remain active for longer (i.e. greater longevity) to increase the probability that sperm will reach and fertilize the remaining eggs (Ball & Parker, 1996; Burness

et al., 2004). Therefore, the 60 s post-activation trial may be relevant for sneaking jack males who may accrue fertilizations through extreme sperm longevity. In addition to sperm competition, we also investigate the role of CFC on fertilization success by examining whether genetic compatibility at the MH class I α_1 and class II β_1 regions can influence competitive fertilization success while also examining MH allele inheritance patterns in resulting offspring. In our study, CFC is examined over time post-sperm activation, where we may expect CFC to be strongest at earlier time points when females have more choices (i.e. when all sperm are active). Therefore, our research quantifies the complex interaction between sperm competition and CFC (i.e. post-copulatory sexual selection), as well as evaluates mechanisms that may contribute to the maintenance of alternative phenotypes in nature. In addition to the evolutionary importance of our study, this work also has practical applications for the propagation and management of the species. The success of hatchery programmes can be improved if breeding protocols are designed in a way that helps maintain genetic variation and mimic natural situations.

Materials and methods

Sperm competition trials

Chinook salmon used in our study were from the Quinsam River, British Columbia, Canada. Gametes were collected from a total of ten males and five females in reproductive condition in October 2003. The males consisted of five mature males ('hooknose males') and five jacks, where jacks were distinguished from hooknose males based on their lack of secondary sexual characteristics, gonad inspection and body size (Heath *et al.*, 2002). Males were dried around the vent and stripped of sperm by applying gentle pressure to the abdomen. Care was taken to avoid contamination with urine, mucous and water during stripping and storage. Spermatocrit (the percentage of the milt volume occupied by the spermatozoa) was measured (Skarstein *et al.*, 2005) for all males, and the volume of sperm used for the fertilization trial was adjusted such that eggs were fertilized with approximately the same number of sperm cells from each male. The jack–hooknose pairs were selected haphazardly, and 2–5 mL of sperm (depending on relative spermatocrit) from each male was mixed and held at hatchery temperature (~10 °C) until fertilization (< 1 h). Eggs were collected by humanely euthanizing the fish and cutting the abdominal wall to release the eggs, and then, eggs from each female were divided into five equal groups, ovarian fluid was drained and eggs were held at hatchery temperature (~10 °C) until equal sperm mixtures were ready for fertilization (1–2 h). Each sire pair (consisting of the mixture of sperm from a paired jack and

hooknose) fertilized one group of eggs from each female to make a 5 × 5 factorial cross with a total of 25 maternal half-sib families. Fertilizations were performed at multiple time points post-activation of sperm to evaluate competitive fertilization success of jack and hooknose males over time since sperm activation to simulate different times spent in the water column prior to contact with the eggs. Therefore, fertilizations were assessed at 0, 15 and 60 s post-activation of sperm in water; however, eggs were not activated prior to exposure to activated sperm. The sperm mixture was activated by adding 20–30 mL of 8 °C hatchery water (four to five times the volume of the sperm mixture) and added to eggs at the appropriate time with additional fresh water (approximately three times the volume of the eggs and sperm mixture). We were not able to measure the volume of the activated sperm mixture added to the eggs at each of the time points; however, the volumes were approximately equal. After fertilization, eggs were incubated separately in a vertical stack incubator. In order to avoid differences in paternity due to viability differences among offspring from the two tactics (see Garcia-Gonzalez, 2008), the experiment was terminated when the eggs developed eyespots (approximately 250 ATU), and 48 eggs were haphazardly selected from each cross at each time point for genetic analysis.

Parentage assignment

DNA was extracted from parental fin clips as well as from embryos dissected from eyed eggs, using a plate-based extraction method (Elphinstone *et al.*, 2003). Parentage was determined for 48 offspring per cross at each time point using previously described microsatellite markers selected to maximize discrimination power among the male parents. Microsatellite markers used were Ots107 (Nelson & Beacham, 1999), OtsG83b (Williamson *et al.*, 2002) and Omy1191UW (Spies *et al.*, 2005). Parental and offspring microsatellite alleles were amplified using polymerase chain reactions (PCR) with fluorescently dye-labelled forward primers that allowed products to be visualized on a LI-COR 4300 DNA analyser. Gene ImagIR software was used to visually score and determine fragment sizes. Alleles were then assigned to the female and either the jack or hooknose males; therefore, paternity was identified by both the exclusion of one sire and positive inclusion of the other sire.

Major histocompatibility genotyping

The PBR of the MH class II β_1 gene and MH class I α_1 gene was PCR amplified from parental samples with primers developed by Miller *et al.* (1997). The PCR profile consisted of: 2-min initial denaturation (95 °C), 30 cycles of 30-s denaturation (95 °C), 30-s annealing

(52 °C), 1-min extension (72 °C), followed by a final 10 min extension (72 °C).

MH PCR products (both MH classes I and II) from parental samples were cloned into a pGEM[®]-T vector following the manufacturer's protocol (Promega, Madison, WI, USA). White colonies were selected, boiled in ddH₂O and then used for insert verification PCR. The insert was amplified using the M13 forward primer (5'-GTA AAA CGA CGG CCA GT-3') and M13 reverse primer (5'-AAA CAG CTA TGA CCA TG-3') under the following conditions: 2-min initial denaturation (94°); 35 cycles of 1-min denaturation (94°), 1-min annealing (55°), 1-min extension (72°); and a final 3-min extension cycle (72°). Each 25 µL reaction consisted of 50–100 ng plasmid DNA, 0.50 µL of each primer (100 ng µL⁻¹), 2.5 µL 10× reaction buffer, 25 mM MgCl₂, 200 µM dNTPs, and 0.50 U AmpliTaq[®] polymerase (Applied Biosystems, Foster City, CA, USA). Eight subclones containing appropriately sized inserts were sequenced from each parent. PCR products were purified using AMPure (Agencourt, Beverly, MA, USA) purification system, and sequencing reactions were performed using the M13 forward primer along with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). After purification using CLEANseq (Agencourt), sequencing was performed using the M13 forward primer and ABI's BigDye Terminator version 3.1 on an ABI 3130xl sequencer.

To evaluate the inheritance of the MH alleles in the F1 generation, a subset of the offspring from each of the 25 maternal half-sib families were genotyped at the MH class I α_1 gene. Offspring were assessed for MH class I inheritance for fertilizations that occurred during the 0 s post-activation time period only, and not later time points. Offspring MH class I α_1 PCR products were directly sequenced, and allelic variation was read from the chromatogram. MH class II β_1 gene lacked sufficient polymorphism among the parents to allow meaningful segregation analysis, and, therefore, offspring were not genotyped at the MH class II β_1 locus.

Statistical analyses

Analysis of reproductive success

All statistical analyses were performed in R statistical software v. 3.1.2 (R Core Team, 2016). The number of jack-sired and hooknose-sired offspring was determined for each family. First, to determine whether the number of jack-sired offspring differed significantly from the number of hooknose-sired offspring at each time point post-activation, generalized linear mixed effect models (GLMMs) with poisson distribution were used with time post-activation, male tactic and their interaction as fixed factors and male, female, their interaction and sire pair as random factors. If a significant interaction between time and tactic was found, *post hoc* slice tests were used to test for determine differences in

fertilization success between tactics at each time point using the lsmeans package (Lenth, 2016). Next, all 48 offspring for each cross and time point were coded as 0 or 1 to represent either hooknose- or jack-sired offspring ($n = 3600$ offspring). Subsequently, we used GLMMs to determine the effect of factors on fertilization success, where time post-activation was a fixed factor with female, sire pair and their interaction as random factors. GLMMs were compared with and without each random and fixed factor to determine their significance in the model. Significance was determined using log-likelihood ratio tests with an alpha level of 0.05.

Analysis of parental MH sequences and MH-mediated cryptic female choice

Geneious Pro software (Biomatters) was used to create alignments to identify alleles. Two alignments, one each for the α_1 and β_1 regions, were built using all sequences from parental samples. Actual alleles occurred at a greater frequency than PCR artefacts, and sequences were verified by comparison to alleles on GenBank and in Helou (2010). Identical sequences were grouped and assigned an allele name.

Next, we determined whether the egg–sperm compatibility was affected by whether male MH genotypes were more similar or dissimilar to the female. Expected offspring α_1 and β_1 genotypes were determined for all parental crosses based on Mendelian segregation. We assumed that MH genotypes follow Mendelian inheritance given that this has been demonstrated in other salmonid studies (Kim *et al.*, 1999; Grimholt *et al.*, 2002; Promerová *et al.*, 2017). As nonrandom inheritance patterns could be possible (see Wedekind *et al.*, 1996; Pitcher & Neff, 2006), we also tested MH allele segregation in offspring as validation for our assumption. Similar to Landry *et al.* (2001), an allele amino acid dissimilarity index (AADI) was estimated for expected offspring genotypes using the number of amino acid differences between the two parental allele sequences, where a higher number would represent greater dissimilarity between alleles. For each maternal half-sib family, the four AADI values were used to determine an average value for the sire and female pair. Next, for each maternal half-sib family, the AADI average obtained from expected hooknose-sired offspring genotypes was subtracted from the AADI average from expected jack-sired offspring (i.e. difference = $AADI_{\text{jack}} - AADI_{\text{hooknose}}$), and this value was used to determine whether difference in AADI was associated with jack fertilization success. If the difference in AADI was positive, this would indicate that the jack was less similar to the female than the hooknose was to that female, whereas a negative value would indicate that the jack was more similar.

Linear mixed effect models (LMMs) were used to determine the relationship between frequency of jack-

sired offspring (paternity) and difference in average AADI. The models included difference in average AADI as a fixed effect with sire pair and female ID as random effects. Analyses were conducted for each post-activation time point (0, 15 and 60 s) for both the α_1 and β_1 genes. LMMs were compared with and without the fixed effect of AADI to determine its significance in the model using log-likelihood ratio tests with an alpha level of 0.05.

Offspring MH allele segregation analysis

Each maternal half-sib family consisted of a female and two possible sires (either the jack or hooknose male); fifteen to 34 offspring were genotyped from each maternal half-sib family for the MH class I α_1 gene. Sequence scanner version 1.0 (Applied Biosystems) was used to view overlapping base peaks in sequences obtained from offspring PCR products for the MH class I α_1 gene. The overlapping peaks identified in offspring sequences were examined and compared to the nucleotide polymorphisms identified in parental sequences to deduce offspring genotype.

Parental alleles are expected to occur at 50% frequency in the offspring based on Mendel's principle of segregation. To determine whether offspring were inheriting paternal MH class I α_1 alleles as expected by Mendel's principle, we used a contingency table to test whether segregation of alleles differed between sire pairs across all females, allowing us to determine whether certain alleles may have an advantage within a sire pair. Next, using a contingency table, we also tested whether the segregation of alleles within a sire pair was dependent on the female, thus allowing us to assess whether segregation is determined by the interaction of egg and sperm. Analyses were conducted for jack- and hooknose-sired offspring separately.

Results

Fertilization success

Although overall fertilization success was not quantified, the proportion of eggs that developed to the eyed stage declined substantially across the three time points post-sperm activation: under the 0 s since activation treatment, close to 100% of the eggs reached the eyed-egg stage while under the 60 s since activation treatment approximately 10–20% of the eggs reached the eyed-egg stage.

Although fertilization success was not directly quantified, we considered paternity at the eyed-egg stage to represent 'relative fertilization success' of males. Relative fertilization success of jack sires at each time point for all half-sib families are presented in Table S1. Using GLMMs, we found a significant interaction between male tactic and time post-activation ($\chi^2 = 370.34$; d.f. = 2; $P < 0.001$), where jack paternity decreased

over time (see Fig. 1). *Post hoc* tests revealed that jacks sired significantly more offspring at 0 s post-activation ($P < 0.001$), and no difference between tactic was found at 15 s ($P = 0.12$); however, hooknose males sired more offspring at 60 s post-activation ($P < 0.001$) (Table S1 and Fig. 1). To further examine factors contributing to jack fertilization success, GLMMs with binomial distribution were used where the response in the models included each egg coded as either 0 or 1 if sired by a jack or hooknose male, respectively. GLMMs included the fixed effect of time with sire pair, female and their interaction as random effects. GLMMs further confirmed that time post-activation had a significant effect on jack fertilization success ($\chi^2 = 380.23$; d.f. = 2; $P < 0.001$). The random factor of female identity did not have a significant effect ($\chi^2 = 0.06$; $P = 0.80$) on jack fertilization success; however, other random effects were significant including sire pair ($\chi^2 = 12.63$; d.f. = 1; $P < 0.001$) and the interaction of sire pair and female ($\chi^2 = 4.03$; d.f. = 1; $P = 0.04$).

MH-mediated cryptic female choice

A total of four MH II β_1 alleles were identified in the parental samples, which included B1-Ots-A, B1-Ots-B, B1-Ots-C and B1-Ots-D, and all nucleotide sequences were found in GenBank (see Table S2 and Appendix S1). Two sire pairs had the same alleles, and four of the five females were homozygous for the same allele (B1-Ots-A). Due to the lack of allelic diversity at this gene in the parental samples, offspring were genotyped for the MH I α_1 gene only, which was found to be more polymorphic, thus making it possible to

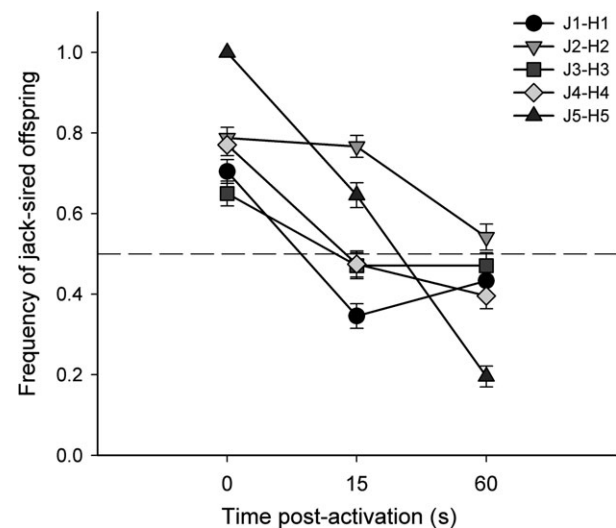


Fig. 1 Mean (\pm standard error) frequency of jack-sired offspring for jack-hooknose (J-H) sire pairs at 0, 15 and 60 s post-activation of sperm. Dashed line represents equal success of jack and hooknose sire under sperm competition.

determine inheritance of maternal and paternal alleles (i.e. allele segregation).

A total of 13 MH I α_1 alleles were identified in the parental samples, and all alleles were found in the GenBank database (see Table S2 and Appendix S2). Translation of the 13 nucleotide sequences revealed that all base substitutions were nonsynonymous, and thus formed distinct amino acid sequences (Table S2). A1-Ots-N, A1-Ots-E and A1-Ots-T were alleles found only in jacks, whereas A1-Ots-D, A1-Ots-C and A1-Ots-I were found only in hooknose males (Table S2). The alleles occurring at the greatest frequency were A1-Ots-P (17.6%), followed by A1-Ots-R (15.7%), A1-Ots-A (12.6%) and A1-Ots-M (12.4%) (see Table S2).

The frequency of jack-sired offspring was not dependent upon the difference in average AADI between jack and hooknose offspring expected genotypes for the MH class I α_1 gene at any post-activation time point (all P values > 0.33 ; see Table 1). The same was true for MH class β_1 gene (all P values > 0.32 ; Table 1).

Major histocompatibility allele segregation in offspring

Next, we analysed the segregation of MH I α_1 alleles in jack- and hooknose-sired offspring. Not all males could be included in the analyses, as one jack and one hooknose (J1 and H3) were homozygous for α_1 alleles, and H5 sired no offspring. Analysis of the segregation of α_1 alleles in offspring revealed no significant difference in allele segregation between sire pairs across all females for both jack- and hooknose-sired offspring (Table S3; all P values > 0.59). Furthermore, we found no significant difference in allele segregation within a sire pair between females for both jack- and hooknose-sired offspring (Table S4, all P values > 0.21). The results of the segregation analysis further validate our decision to use expected offspring MH genotypes to calculate allele divergence between parents (AADI) for cryptic female choice analyses.

Discussion

In our study, upon initial sperm activation, jack sperm outcompeted hooknose sperm and fertilized the majority of the eggs in all 25 maternal half-sib families. The elevated competitive fertilization success of jacks is not surprising given that studies on many salmonid species, including Chinook salmon (Flannery *et al.*, 2013; Young *et al.*, 2013; Lehnert *et al.*, 2017), have shown that sperm from precociously maturing males have a competitive advantage compared to sperm from the guarding male phenotype. Similar findings have been observed in bluegill sunfish (*Lepomis macrochirus*), sticklebacks (Gasterosteidae) and in other salmonid species (de Fraipont *et al.*, 1993; Gage *et al.*, 1995; Vladic & Jarvi, 2001; Stoltz & Neff, 2006). We also found a highly significant jack–hooknose sire pair effect on jack fertilization success, which likely reflects differences in relative sperm swimming velocity between jack and hooknose sire pairs. In Chinook salmon, sperm swimming velocity is significantly faster in jacks compared to hooknose males at 5-s post-activation (Flannery *et al.*, 2013), thus indicating jacks may have evolved faster-swimming sperm to reach the egg faster and compete directly with hooknose sperm for fertilization. Alternatively, the interaction of jack and hooknose semen may influence male fertilization success. It has recently been demonstrated that jack seminal plasma can reduce hooknose sperm velocity in Chinook salmon; however, hooknose seminal plasma does not influence jack sperm velocity (Lewis & Pitcher, 2017). This interaction between tactics could provide another mechanism that could explain the increased fertilization success of jacks demonstrated in our study. Our study provides evidence that jacks have evolved means to compensate for their subdominant position in natural spawning events, thus highlighting that sexual selection mechanisms may operate to maintain both tactics in nature.

We also found a significant and substantial effect of time since sperm activation on jack fertilization success.

Table 1 Results from linear mixed effect models analyses testing the relationship between frequency of jack-sired offspring and the difference in the average amino acid dissimilarity index (AADI) at MH I α_1 and MH II β_1 between jack and hooknose per maternal half-sib family.

Genetic predictor (fixed effect)	Time post-activation (s)	Variance of random effects and error in model			Fixed effect	
		J–H sire pair	Female	Error	χ^2 (d.f. = 1)	P -value
MH I α_1	0	0.018	0.000	0.003	0.89	0.35
	15	0.029	0.001	0.004	0.96	0.33
	60	0.012	0.000	0.025	0.35	0.56
MH II β_1	0	0.015	0.000	0.003	0.81	0.37
	15	0.027	0.001	0.004	0.13	0.71
	60	0.013	0.000	0.024	0.69	0.41

Linear mixed effect models were performed for each genetic predictor (fixed effect) at three time points post-activation including 0, 15 and 60 s and included the random effects of jack–hooknose (J–H) sire pair and female ID. Variance associated with random effects and residual error are presented.

Jacks sired the majority of offspring in all crosses when fertilization occurred at 0 s post-activation; however, jack paternity declined over time where hooknose males sired significantly more eggs overall at 60 s post-activation. Our study is the first to demonstrate that competitive fertilization success changes over time post-activation. A possible explanation for the change in paternity over time is a trade-off between sperm swimming speed and sperm longevity (Ball & Parker, 1996; but see Flannery *et al.*, 2013). In bluegill sunfish, sneaker males have faster sperm initially; however, the motility of sperm declines more rapidly for sneaker males relative to parental males (Burness *et al.*, 2004). Faster initial sperm velocity may be an especially important mechanism allowing jack males to achieve fertilization success in nature (see Hoysak & Liley, 2001).

We found no significant effect of female identity on competitive fertilization success; however, we did find a significant female \times sire-pair interaction effect indicating evidence of cryptic female choice (CFC). In salmonids, CFC may be mediated by the egg and/or ovarian fluid characteristics (Lahnsteiner, 2002; Rosengrave *et al.*, 2009, 2016; Yeates *et al.*, 2009, 2014; Butts *et al.*, 2012b, 2017; Gessner *et al.*, 2017a, b). Although ovarian fluid can influence the outcome of sperm competition between jack and hooknose males (see Butts *et al.*, 2017; Lehnert *et al.*, 2017), ovarian fluid was removed from the eggs in our study and therefore evidence of CFC may only be explained by egg-sperm interactions. We also found that variation in fertilization success due to egg-sperm interactions could not be explained by divergence at the MH I or II PBRs. Analysis of the MH class I α_1 gene region revealed high allelic polymorphism, whereas the MH class II β_1 gene region showed lower diversity, as found in other Chinook salmon populations (Miller *et al.*, 1997; Pitcher & Neff, 2006). Although our study found no relationship between fertilization success and divergence at either MH gene region, a few studies in salmonids have demonstrated a relationship between MH divergence and *in vitro* competitive fertilization success. For example, Gessner *et al.* (2017a) recently found that fertilization success in Chinook salmon was related to divergence at the MH II gene (but not the MH I gene), where less divergent males attained higher competitive fertilization success, and the same has been demonstrated in guppies (*Poecilia reticulata*) (Gasparini *et al.*, 2015). Although similar results have been found at the MH I gene in Atlantic salmon (*Salmo salar*) (Yeates *et al.*, 2009), there are also *in vitro* competitive fertilization studies that have failed to find evidence of MH-mediated CFC in salmon. For example, in Chinook salmon, Lehnert *et al.* (2017) found no relationship between MH II divergence and *in vitro* competitive fertilization success. In Arctic charr (*Salvelinus alpinus*), whereas male *in vitro* competitive fertilization success was related to MH II heterozygosity,

there was no relationship with MH II divergence (Skarstein *et al.*, 2005).

In addition to measuring the influence of MH divergence on competitive fertilization success, we also quantified the segregation of MH I α_1 alleles into the offspring of jacks and hooknose males. Inheritance patterns of MH I α_1 alleles did not deviate from Mendelian inheritance, consistent with previous MH inheritance studies in salmonids (Kim *et al.*, 1999; Grimholt *et al.*, 2002; Promerová *et al.*, 2017). Recently, Promerová *et al.* (2017) conducted *in vitro* fertilizations using single males (i.e. no interindividual sperm competition) to examine the role of nonrandom gamete fusion in post-copulatory choice at the MH II gene in Atlantic salmon. Promerová *et al.* (2017) found no evidence of nonrandom gamete fusion or allele transmission distortion suggesting that if CFC for MH II is occurring in salmon (as found only by Gessner *et al.*, 2017a to date), it is likely acting on diploid genotypes rather than at the allelic level.

Whether CFC operates on the MH genes in salmonids warrants further investigation given the conflicting published evidence; however, we should acknowledge that our study differs from studies that have found evidence of CFC effects on MH genes, as our study incorporates two alternative male reproductive tactics (as does Lehnert *et al.*, 2017). Although our sample size is limited ($n = 15$ adults), our design resulted in 25 half-sibling families and our results still revealed that CFC is occurring at the male \times female level. Here, we propose that CFC may not simply operate via the MH gene(s) alone because MH 'choosy' eggs may be at risk of remaining unfertilized. Thus, CFC mediated solely by MH genes could be a costly strategy for salmonids, given the limited time that eggs and sperm remain active. Instead, CFC may operate based on several components of genetic variation and gamete signalling, thus complicating the relationship examined here when multiple levels of genetic differences exist between competing males. With the advancement of next-generation sequencing technology, we propose that future studies should not only measure MH divergence, but also incorporate several measures of genetic/genomic variation and consider other genes related to local adaptation, mate choice and/or post-copulatory selection. For example, recent work in Chinook salmon found that male-female relatedness at different genomic regions (independent of genomewide relatedness) was associated with sperm-ovarian fluid interactions as well as egg-sperm interactions, underscoring the power of genomics in post-copulatory studies (Gessner *et al.*, 2017b). Further, we should acknowledge the possibility that female choice (pre- and post-copulatory) may vary temporally and spatially, and this may explain some discrepancies among studies. For example, in Atlantic salmon, evidence from a natural spawning population suggests that mate choice can differ between MH genes

and between years (Tentelier *et al.*, 2017). Altogether, it seems clear that CFC is operating in salmonids; however, future work is needed to address the mechanisms driving this process in salmon, such as identifying what variation CFC is acting upon and how the egg itself can facilitate choice.

Apart from the evolutionary importance of our study, our research also has significant implications for hatchery and aquaculture practices. The outcome of sperm competition in salmonids is largely dependent on sperm traits such as sperm velocity (Gage *et al.*, 2004), and here, we provide evidence for a bias towards fertilization success in a specific male life history under *in vitro* sperm competition. Given that there is evidence for a strong additive genetic component to jacking rates (Heath *et al.*, 1994), one would expect that selection for jacks would occur over a relatively short period of time under hatchery conditions. Therefore, hatcheries should attempt to mimic natural spawning, and one way in which hatcheries could provide more natural conditions during fertilization would be by including ovarian fluid in fertilization protocols, as ovarian fluid has been reported to allow both tactics to attain similar success during *in vitro* competitive fertilization (Butts *et al.*, 2017; Lehnert *et al.*, 2017). Hatcheries play a crucial role in the management and conservation of salmon; therefore, it is of great importance that more genetically benign management strategies are implemented (Camp-ton, 2005).

In conclusion, our study found differences in male fertilization success between alternative reproductive tactics in Chinook salmon, likely mediated by a combination of differences in sperm quality and, to a lesser degree, CFC. In our study, a significant interaction (female \times sire pair) effect can only be explained by CFC mediated by the egg itself (i.e. egg choice); however, we found that this was not related to divergence at the MH I and II genes. Our study is the first to have analysed the relationship between post-copulatory sexual selection and MH-mediated CFC over time post-activation in alternative reproductive tactics; thus, our work provides novel insights into the evolutionary mechanisms of sperm competition and CFC occurring in alternate male phenotypes. Our results suggest that jacks have evolved mechanisms to counteract their subordinate spawning position in nature, and thus suggest that mechanisms of sexual selection contribute to the maintenance of both tactics in nature. These mechanisms have important evolutionary as well as management implications, which with further research will be valuable tools for predicting population dynamics and implementing management strategies.

Ethics

The fieldwork in this study followed all Federal and local animal care requirements.

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Authors' contributions

D.D.H., T.E.P., J.W.H. and L.H. designed the research and generated the data set; L.H. and S.J.L. analysed the data and drafted the manuscript. In addition, all authors contributed to the writing of the manuscript and gave final approval for publication.

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Competing interests

We have no competing interests.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1 Proportion of eyed-eggs sired by jack Chinook salmon (*Oncorhynchus tshawytscha*) males under *in vitro* sperm competition with hooknose males.

Table S2 Alleles identified in the Chinook salmon study population for the MH class I α_1 and MH class II β_1 gene.

Table S3 Contingency table of segregation of major histocompatibility class I (MH I) α_1 alleles in jack- and hooknose-sired Chinook salmon (*Oncorhynchus tshawytscha*) offspring within sire-pairs across all five females.

Table S4 Contingency tables of segregation of major histocompatibility class I (MH I) α_1 alleles in jack- and hooknose-sired Chinook salmon (*Oncorhynchus tshawytscha*) offspring between females within sire-pairs.

Appendix S1 The nucleotide sequences of the 4 MH class II β_1 alleles found in the study population of Chinook salmon (*Oncorhynchus tshawytscha*). Nucleotide differences are indicated in grey.

Appendix S2 The nucleotide sequences of the 13 MH class I α_1 alleles found in the study population of Chinook salmon (*Oncorhynchus tshawytscha*). Nucleotide differences are indicated in grey.

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