Conserv Genet (2010) 11:2333–2342
DOI 10.1007/s10592-010-0119-3

RESEARCH ARTICLE

MHC-mediated local adaptation in reciprocally translocated Chinook salmon

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Received: 27 October 2009 / Accepted: 2 August 2010 / Published online: 19 August 2010
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Abstract Most Pacific salmonid populations have faced significant population declines over the past 30 years. In order to effectively conserve and manage these populations, knowledge of the evolutionary adaptive state of individuals and the scale of adaptation across populations is needed. The vertebrate major histocompatibility complex (MHC) represents an important adaptation to parasites, and genes encoding for the MHC are widely held to be undergoing balancing selection. However, the generality of balancing selection across populations at MHC loci is not well documented. Using Chinook salmon (*Oncorhynchus tshawytscha*) from two populations, we follow the survival of full-sib family replicates reared in their natal river and reciprocally transplanted to a foreign river to examine selection and local adaptation at the MHC class I and II loci. In both populations, we found evidence of a survivorship advantage associated with nucleotide diversity at the MHC class I locus. In contrast, we found evidence that MHC class II diversity was disadvantageous in one population. There was no evidence that these effects occurred in translocated families, suggesting some degree of local adaptation at the MHC loci. Thus, our results implicate balancing selection at the MHC class I but potentially differing selection across populations at the class II locus.

Keywords Major histocompatibility complex · Chinook salmon · Adaptive divergence · Nucleotide divergence · Balancing selection · Directional selection · Survival

Electronic supplementary material The online version of this article (doi:10.1007/s10592-010-0119-3) contains supplementary material, which is available to authorized users.

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Introduction

Most Pacific salmonid (*Oncorhynchus* spp.) populations have suffered significant population declines over the last 30 years (Nehlsen et al. 1991). These declines have been caused by a variety of factors including overfishing, habitat loss, interactions with invasive species (Nehlsen et al. 1991), and more recently, evidence suggests that parasites also play an important role in population declines (Dobson and Foufopoulos 2001; Harvell 2004). Thus, in order to conserve and manage endangered salmonid populations, detailed knowledge of local adaptations driven by environmental variation in parasite communities is required. The major histocompatibility complex (MHC) represents the interface between the vertebrate immune system and the parasite community and thus, is expected to be undergoing natural selection from variation in the parasite community. The MHC encodes for proteins that are involved in the activation of an immune response through the recognition and presentation of pathogen-derived peptides to T-cells (Klein 1986). MHC class I molecules generally function in the recognition of intracellular

peptides derived from viruses, whereas the MHC class II functions in the recognition of extracellular peptides derived from macroparasites and bacteria (Klein 1986; Nei and Hughes 1991). The MHC has been shown to display considerable genetic variation within and among populations in many vertebrates including birds, mammals, and fishes (e.g. Wegner et al. 2003; Bryja et al. 2007; Ekblom et al. 2007; Evans et al. 2010) and this diversity is likely to have arisen in response to variation in the pathogen community (Apanius et al. 1997).

It has been proposed that selection, and ultimately local adaptation, at the MHC occurs through several mechanisms (Bernatchez and Landry 2003). Balancing selection has been proposed as the most likely mechanism maintaining the high degree of genetic variation within and among populations. Balancing selection may operate through a heterozygote advantage, in which case more genetically diverse individuals are able to mount an immune response against a wider range of pathogens than homozygous individuals (Doherty and Zinkernagel 1975; e.g. Hedrick et al. 2001; Arkush et al. 2002; McClelland et al. 2003, Evans and Neff 2009). Several studies have found that MHC class II heterozygotes exhibit lower parasite loads than homozygotes (e.g. Hedrick et al. 2001; Froeschke and Sommer 2005; see Wegner et al. 2004 for a review) or that there is an excess of heterozygotes within populations, which suggests a selective advantage for heterozygotes (e.g. Peters and Turner 2008; Oliver et al. 2009; Evans et al. 2010; see Apanius et al. 1997; Garrigan and Hedrick 2003 for reviews). Furthermore, individuals bearing more divergent MHC alleles may be able to recognize a broader range of pathogens than individuals bearing more similar MHC alleles (Wakeland et al. 1990). Conversely, MHC loci can undergo directional selection when a particular allele confers a strong selective advantage such as resistance to a pervasive parasite (Blanchet et al. 2009; Fraser and Neff 2010). Thus, the degree of local adaptation exhibited across populations will depend on the strength and form of selection operating within each population. However, despite a growing interest in the evolution and maintenance of MHC genetic variation, the generality and relative importance of balancing versus directional selection across populations remains unclear (Bernatchez and Landry 2003).

Pacific salmonid populations represent an ideal system in which to investigate the mechanisms of selection and the extent of local adaptation on MHC genes as populations occur across a broad geographic gradient and face highly diverse environments (Taylor 1991). Salmonids express a high degree of natal philopatry, in which the majority of individuals return to the river from where they were born to reproduce (Groot and Margolis 1991; Hendry et al. 2004), and this behaviour is likely to minimize the loss of locally adaptive changes through gene flow (Taylor 1991). Our study focuses on the Quinsam River and Big Qualicum River populations of Chinook salmon (*O. tshawytscha*) located in British Columbia, Canada. The populations are located on the east coast of Vancouver Island and are separated by approximately 100 km. Population divergence between the Big Qualicum and Quinsam rivers is higher at the MHC class I and lower at the MHC class II than expected under neutrality, which suggests that these populations are undergoing local adaptation through potentially differing forms of selection (Evans et al. 2010). Indeed, Chinook salmon juveniles in the Quinsam and Big Qualicum rivers may face differing levels of infections by a highly diverse bacterial pathogen community (Evans and Neff 2009). Thus, pathogen communities could be a strong source of selection on the MHC in these populations.

Here we follow Chinook salmon family groups from the Big Qualicum and Quinsam populations during the freshwater life-history stages in order to investigate the role of MHC nucleotide diversity on survival. Individuals were reared in a common garden experiment within a hatchery environment, so survival was not affected by predation or limited by food availability. The families were exposed to untreated river water, which can be an important source of selection through pathogen exposure (Amos and Thomas 2002, Wedekind et al. 2010). We test the null hypothesis that juvenile survival is random with respect to MHC similarity between parents. Previous studies have indicated that the onset of MHC class I and II gene expression may vary across juvenile development (Rodrigues et al. 1998). As such, we examined selection on the MHC loci separately during the embryo, larvae, and fry developmental stages. Then, using reciprocal translocations between populations, we experimentally challenge families by rearing them in a non-natal environment in order to examine local adaptation at the MHC.

Materials and methods

Crosses

We generated families by conducting all possible crosses between eight males and eight females in each population. Crosses were conducted on October 8, 2007 at the Fisheries and Oceans Canada Big Qualicum River hatchery located near Qualicum Beach, B.C., and on October 15, 2007 at the Fisheries and Oceans Canada Quinsam River hatchery located near Campbell River, B.C. We collected Chinook salmon adults from the Big Qualicum River using diversion channels located at the Big Qualicum hatchery. Adults were collected via seine netting from natural holding ponds in the Quinsam River. Males and females were euthanized

using carbon dioxide in order to collect milt from males and eggs from females. A total of 64 unique families were generated within each population. Each cross was replicated four times ($N = 256$ "family replicates") and each replicate consisted of 100 eggs. Family replicates were stored in individual egg tubes (Dynamic Aqua-supply Ltd, Surrey, Canada) and were randomly allocated to a location within a Heath incubation tray system. Offspring were exposed to natural river water for the duration of their development.

Reciprocal translocations

We conducted translocations between each population by moving half of the replicates from each family (two replicates per family; $N = 128$ replicates) to the reciprocal hatchery (i.e. Quinsam to Big Qualicum and Big Qualicum to Quinsam). Translocations were performed when the Chinook salmon developing embryos became "eyed", at which point they are no longer sensitive to light exposure and movement (Stead and Laird 2002). Control family replicates that remained in the natal hatchery were also handled and moved so as to replicate the translocation.

Embryo and larvae survival

When the embryos became eyed, families were monitored weekly for offspring survival. We initially assessed survival at the eyed stage, hereafter referred to as "embryo survival", for each family replicate as the proportion of survivors out of 100. For comparisons between control and translocation family replicates, survival from the time of translocation to the end of the larvae stage was used. This "larvae survival" estimate was calculated as # survivors at end of larvae stage/# survivors at translocation. We were unable to assay the specific causes of mortality as part of this study, but viral, bacterial, and fungal pathogens have been shown to cause mortality in salmon hatcheries (Bruneau et al. 1999; Amos and Thomas 2002, Wedekind et al. 2010).

Fry survival

In order to continue rearing family replicates through the fry (endogenous feeding) stage, it was necessary to pool all 64 families within a replicate, ensuring that individual replicates were reared separately. Fry were reared in Capilano troughs and were fed EWOS feed (EWOS Canada Ltd., Surrey, B.C.) ad libitum. The fry were reared until April 2, 2008, which is about the time of normal ocean migration for these populations. Thus, translocated offspring were reared for a total of 134 days in the non-natal environment. Two hundred fry from each replicate (i.e. 400 control fry and 400 translocated fry from each population) were then randomly sampled and a small tissue sample was obtained. The tissue samples were stored in 95% ethanol.

Microsatellite parentage analysis

To obtain an estimate of fry survival for each family we used microsatellite parentage analysis. We used the DNA Wizard Extraction kit (Promega Corp., Madison, WI, USA) to isolate DNA from the tissues of parents and offspring. Chinook salmon parents and fry were genotyped at eight microsatellite loci (Table 1) and loci were amplified using

Table 1 Microsatellite loci used to assess parentage of Chinook salmon (*Oncorhynchus tshawytscha*) fry in the Quinsam and Big Qualicum rivers

Locus	Primer sequence[a]	T_m (°C)	Big Qualicum			Quinsam		
			H_O	H_E	A	H_O	H_E	A
Ots107	F-ACAGACCAGACCTCAACA R-ATAGAGACCTGAATCGGTA	58	16	15.0	14	16	14.6	11
Ots104	F-GCACTGTATCCACCAGTA R-GTAGGAGTTTCATTTGAATC	64–49[b]	15	14.9	16	13	13.6	12
OtsG311	F-GCGGTGCTCAAAGTGATCTCAGTCA R-TCCATCCCTCCCCCATCCATTGT	64–49[b]	15	14.0	18	16	15.2	16
OtsG249	F-TCTCAGAGGGTAAAATCTCAGTAAG R-GTACAACCCCTCTCACCTACCC	64–49[b]	16	15.1	15	15	15.3	18
Omy325	F-TGTGAGACTGTCAGATTTTGC R-CGGAGTCCGTATCCTTCCC	56	13	13.2	8	10	11.4	6
OtsG83B	F-TAGCCCTGCACTAAAATACAGTTC R-CATTAATCTAGGCTTGTCAGCAGT	60	15	15.1	15	16	14.9	13
OtsG68	F-CATGTACGTGGCGAAGCCCTC R-CATGTCGGCTGCTCAATGTA	55	16	15.3	16	13	14.1	14
OtsG432	F-TGAAAAGTAGGGGAAACACATACG R-TAAAGCCCATTGAATTGAATAGAA	64–49[b]	13	11.9	2	8	10.9	10

For each microsatellite, forward and reverse primer sequences, PCR annealing temperatures (T_m), observed (H_O) and expected heterozygosity (H_E) and number of alleles found in the 16 parents from each population are indicated

[a] Primer sequences published in Nelson and Beacham (1999), Williamson et al. (2002), O'Connell et al. (1997)

[b] Touchdown PCR: Annealing temperature set at 64°C for initial cycle, then dropped by 1°C in each subsequent cycle until a final annealing temperature of 49°C was reached, which was continued through the remaining cycles

polymerase chain reaction (PCR) following the protocol outlined in Heath et al. (2006). Each forward primer was tagged with a fluorescent dye, which enabled the PCR products to be compared against size standards using the Li-Cor 4300 DNA analyzer (Li-Cor Biosciences, Lincoln, Nebraska). We calculated the number of alleles and the expected (H_E) and observed (H_O) heterozygosity at each microsatellite locus in Genepop v. 3.4 (Raymond and Rousset 1995). Parentage was assigned using the program CERVUS v. 3.0 (Kalinowski et al. 2007). Only offspring with genotypes at five or more loci were included in the analysis. For each pooled replicate, offspring were assigned to a family (parent pair) using a 95% confidence criterion implemented in CERVUS. We were unable to enumerate all of the fry in the troughs at the end of the experiment, so we calculated an index of "relative fry survival" for each family replicate. Relative fry survival was calculated by dividing the proportion of genotyped offspring identified to a family replicate by the proportion of offspring from that family replicate that were introduced into the tank. A value of less than one would indicate a family with lower than average survival, and a value of greater than one would indicate a family with higher than average survival as compared to all families introduced into a trough.

We also used the microsatellite data to calculate multilocus heterozygosity (MLH), the proportion of heterozygous loci across all loci genotyped, for each parent. We examined the contribution of MLH to offspring survival through the embryo, larvae, and fry stages using multiple linear regression, with sex and population included as factors in the model, and these results are reported in the Supplemental Materials (Table S1).

MHC genotyping

For each parent, we examined genetic variation within a portion of the MHC class I-A1 and MHC class II-B1 loci using primer sets described in Grimholt et al. (1993), Hordvik et al. (1993). Primers were designed to amplify exons encoding the peptide binding region (PBR) located on the α_1 chain of the MHC class I and the β_1 chain of the MHC class II. PCR was used to amplify each locus following the protocols outlined in Miller et al. (1997) and Docker and Heath (2002). For both loci, PCR products were visualised using SSCP (single strand conformation polymorphism) electrophoresis on the Amersham-Biosciences SSCP system and gels were fixed and stained using a silver stain (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). We cloned samples from each unique conformation using the Promega pGEM T-easy vector kit (Promega Corp., Madison, WI, USA). Between four and eight colonies containing inserts were sequenced for each unique conformation. Alleles that were amplified only once were verified by retyping the individuals exhibiting the allele. Amplification products were sequenced by Genome Quebec (McGill University, Montreal, Canada). Sequences were aligned using Molecular Evolutionary Genetics Analysis (MEGA) software 4 (http://www.megasoftware.net/, Tamura et al. 2007) and chromatograms were read in FinchTV (Geospiza Inc., Seattle, WA, USA, http://www.geospiza.com). MHC class I sequences were aligned to the human HLA-A1 (Bjorkman et al. 1987) and MHC class II sequences were aligned to the human HLA DRB-1 (Brown et al. 1993). These alignments were used to identify the nucleotides that encode the putative PBR—the nucleotides directly involved in the binding of pathogens—in Chinook salmon based on the known PBR in the human HLA. Sequences were also aligned to previously reported Chinook salmon MHC class I-A1 or II-B1 alleles (Evans et al. 2010).

MHC nucleotide divergence

Nucleotide divergence at MHC loci was examined for each parent pair, as it is predicted that diversity in MHC alleles will increase offspring ability to respond to infection by multiple parasites (Wakeland et al. 1990). We calculated the nucleotide divergence (D_{xy}) for each parent pair by summing the pairwise nucleotide distances between all MHC allele combinations between the parents (i.e. Parental alleles = A, a, and B, b; $D_{xy} = D_{AB} + D_{Ab} + D_{aB} + D_{ab}$; Landry et al. 2001). These calculations were conducted separately for the MHC class I and class II. We also calculated the divergence between MHC alleles using only the nucleotides that encode for the PBR of each MHC molecule, as it is predicted that this region in particular should be the subject of diversifying selection (Landry et al. 2001). The results for the two D_{xy} measures were similar, so we present only those based on the PBR. The results based on the whole MHC sequence are reported in the Supplemental Materials (Table S2).

Non-random offspring survival

We used Monte Carlo simulations to examine whether survival of offspring through the embryo, larvae, and fry stages was non-random with respect to MHC class I and class II nucleotide divergence between parent pairs (see Manly 1997). Each family replicate was included as an individual data point in the simulations. During the larvae and fry stages, we used separate simulations for control and translocation family replicates. Our null hypothesis was that survival would be random with respect to MHC nucleotide divergence among parents in both control and translocation treatments. Briefly, the Monte Carlo

simulation randomly assigns observed survival rates, with replacement, across families and then calculates the mean MHC nucleotide divergence across the pseudo-families. The routine was repeated for a total of 1,000 simulations to generate a distribution of mean MHC divergence values expected under the null hypothesis. The distribution of expected values was then compared to the mean observed MHC divergence to determine whether the observed divergence differs significantly from that expected under random survival. The proportion of the expected values falling above or below the observed value was used to calculate the significance of the difference and whether the null hypothesis could be rejected. The simulations were repeated a second time to confirm the significance of the difference between observed and expected values. For each simulation examining the observed and expected MHC class I D_{xy} and MHC class II D_{xy} in our two populations, we used an adjusted critical alpha level of 0.005 ($\alpha = 0.05/10$) to account for the multiple comparisons conducted on embryo, larvae (control and translocation), and fry (control and translocation) survival.

Results

A total of 19,291 and 24,020 embryos survived to the eyed stage in the Big Qualicum and Quinsam populations, respectively. Embryo survival across family groups ranged between 0 and 100% (mean = 77 ± 25%; median = 86%) in the Big Qualicum population and 62–100% (mean = 94 ± 8%; median = 96%) in the Quinsam population. A total of 9,400 and 12,005 offspring survived through the larvae stage in the Big Qualicum and Quinsam control treatments, respectively. Across family groups larvae survival ranged between 70 and 100% (mean = 98 ± 5%; median = 100%) for the Big Qualicum control family replicates and 94–100% (mean = 99 ± 1%; median = 100%) for the Quinsam River control family replicates. A total of 9,488 and 11,877 offspring survived through the larvae stage in the Big Qualicum and Quinsam translocation treatments, respectively. Across the Big Qualicum translocation family replicates, larvae survival ranged between 0 and 100% (mean = 97 ± 10%; median = 85%), whereas it ranged between 71 and 100% (mean = 99 ± 3%; median = 100%) across Quinsam River translocation family replicates.

A total of 681 fry from the Big Qualicum family replicates and 688 fry from the Quinsam River family replicates were identified to a parent pair using microsatellite genotyping. The relative fry survival in Big Qualicum and Quinsam control family replicates ranged between 0 and 9.74 and 0 and 3.44, respectively. The relative fry survival for the translocation replicates ranged between 0 and 5.57 for Big Qualicum family replicates and between 0 and 3.22 for the Quinsam River family replicates. In all four cases the mean relative survival was 1.0 based on our defined calculation.

MHC and microsatellite diversity

A total of 11 MHC class I alleles and four MHC class II alleles were isolated from the Big Qualicum adults (Table 2). Nine MHC class I and four MHC class II alleles were isolated from the Quinsam adults. The eight microsatellite loci were highly polymorphic, with between 6 and 18 alleles observed per locus (Table 1).

MHC and offspring survival

Embryo survival prior to translocation

The results of the Monte Carlo simulations are summarized in Table 3 and in the Supplemental Materials (Figs. S1–S3). During the embryo stage of development, in both the Big Qualicum and Quinsam populations, the mean observed MHC class I nucleotide divergence was significantly higher

Table 2 MHC allele frequencies in Chinook salmon (*Oncorhynchus tshawytscha*) parents from the Big Qualicum and Quinsam rivers

	Big Qualicum	Quinsam
MHC class I Allele		
2	0.06	0.09
3	0.06	0.56
4	0	0.03
5	0.03	0.03
6	0.09	0.03
7	0.09	0.06
9	0	0.06
10	0.06	0.06
12	0.31	0
14	0	0.06
20	0.06	0
23	0.03	0
25	0.06	0
29	0.13	0
MHC class II Allele		
2	0.59	0.88
3	0.25	0.06
4	0	0.03
7	0.13	0
8	0.03	0.03

NB. Sequences of each allele can be found in Evans et al. (2010)

Table 3 Summary of the results of the Monte Carlo simulations for tests of non-random offspring survival with respect to nucleotide divergence (D_{xy}) within the MHC peptide-binding region for two populations of Chinook salmon (*Oncorhynchus tshawytscha*)

Population	Stage		MHC class I D_{xy}	MHC class II D_{xy}
Big Qualicum	Embryo		**+ (0.0001)**	+ (0.09)
	Larvae	Control	+ (0.08)	+ (0.84)
		Translocation	+ (0.01)	+ (0.87)
	Fry	Control	+ (0.35)	+ (0.06)
		Translocation	+ (0.64)	+ (0.52)
Quinsam	Embryo		**+ (0.004)**	+ (0.13)
	Larvae	Control	+ (0.01)	**− (0.0001)**
		Translocation	+ (0.20)	+ (0.74)
	Fry	Control	+ (0.56)	− (0.05)
		Translocation	+ (0.92)	+ (0.38)

Observed values for MHC D_{xy} that were greater than or less than the mean expected value based on an assumption of random offspring survival are indicated, respectively by the + and − symbols. The p-value associated with the difference is shown in parentheses. Values falling below the set critical alpha of 0.005 are shown in *bold*. Control families remained in their natal environment whereas translocation families were reared in the reciprocal hatchery

than expected based on the null hypothesis of random embryo survival (Big Qualicum: mean observed D_{xy} = 14.40, $P = 0.0001$; Quinsam: mean observed D_{xy} = 9.23, $P = 0.004$; Fig. 1a,b). The MHC class II nucleotide divergence was not significantly different than expected based on random embryo survival in either population (Big Qualicum: mean observed D_{xy} = 5.24, $P = 0.09$; Quinsam: mean observed D_{xy} = 2.19, $P = 0.13$).

Larvae survival following translocation

In the Big Qualicum population, nucleotide divergence was not significantly different than expected based on random larvae survival at either the MHC class I in the control replicates (mean observed D_{xy} = 17.99, $P = 0.07$) or at the MHC class II in either the control replicates (mean observed D_{xy} = 6.74, $P = 0.84$) or translocation replicates (mean observed D_{xy} = 6.64, $P = 0.87$). However, in the control family replicates from the Quinsam population and the translocation family replicates from the Big Qualicum population, there was a trend towards higher nucleotide divergence at the MHC class I, though using our adjusted critical alpha, the differences were not significant (Quinsam: mean observed D_{xy} = 9.64, $P = 0.01$; Big Qualicum: mean observed D_{xy} = 17.92, $P = 0.01$). However, nucleotide divergence at the MHC class II was significantly lower than expected in the Quinsam control family replicates (mean observed D_{xy} = 2.27, $P = 0.0001$; Fig. 1c). The translocation replicates from the Quinsam population did not differ from expectations of random survival at either MHC locus (class I: mean observed D_{xy} = 9.64, $P = 0.20$; class II: mean observed D_{xy} = 2.28, $P = 0.74$).

Relative fry survival

During the fry stage, observed MHC class I nucleotide divergence was not significantly different than expected under random survival in either the Big Qualicum population (control replicates: mean observed D_{xy} = 18.67, $P = 0.35$; translocation replicates: mean observed D_{xy} = 18.23, $P = 0.64$) or the Quinsam population (control replicates: mean observed D_{xy} = 9.65, $P = 0.56$; translocation replicates: mean observed D_{xy} = 9.23, $P = 0.92$). Similarly, observed MHC class II nucleotide divergence was not significantly different than expected under random offspring survival during the fry stage in the Big Qualicum population (control replicates: mean observed D_{xy} = 7.34, $P = 0.06$; translocation replicates: mean observed D_{xy} = 6.85, $P = 0.52$) or the Quinsam translocation family replicates (mean observed D_{xy} = 2.38, $P = 0.38$). However, there was a trend towards lower nucleotide divergence in the Quinsam control family replicates (mean observed D_{xy} = 1.96, $P = 0.05$).

Discussion

Many studies support the role of balancing selection in the maintenance of genetic variation at the MHC (Bernatchez and Landry 2003; Piertney and Oliver 2006). In this study, we followed the survival of Chinook salmon juveniles from two populations to test the MHC nucleotide diversity hypotheses. Our results provide strong support for a nucleotide diversity advantage at the MHC class I during the embryo stage in both the Big Qualicum and Quinsam populations. There was, however, only marginal support for a nucleotide diversity advantage during the larvae stage

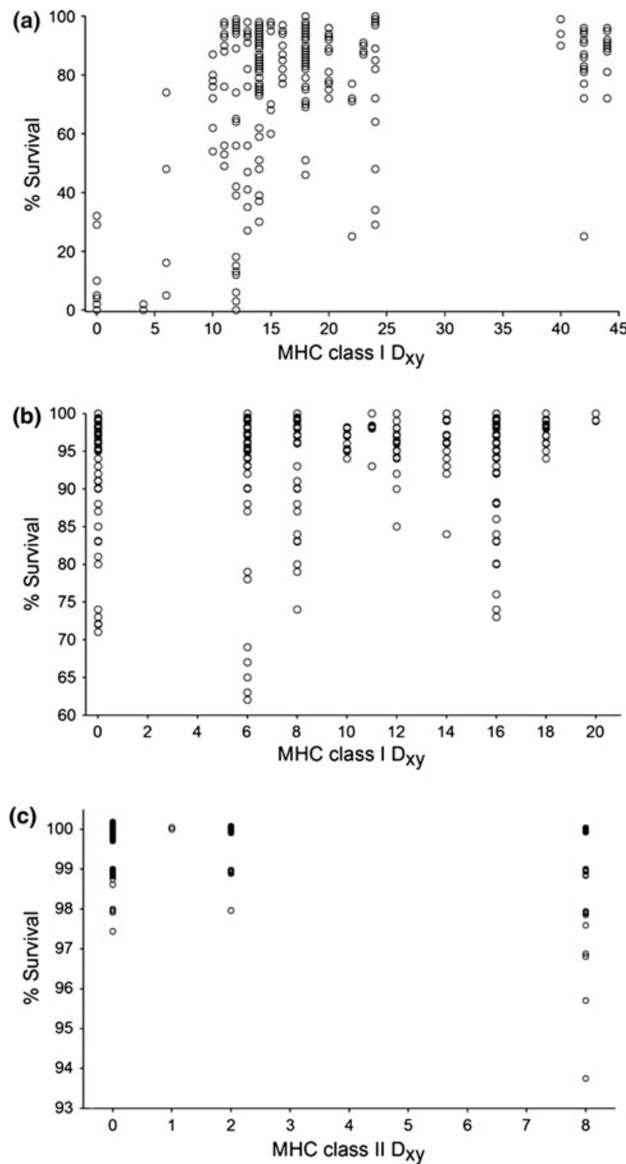

Fig. 1 MHC nucleotide divergence and offspring survival in Chinook salmon (*Oncorhynchus tshawytscha*). Relationships between **a** Big Qualicum and **b** Quinsam embryo survival and MHC class I nucleotide divergence and **c** Quinsam control larvae survival and MHC class II nucleotide divergence are shown. Nucleotide divergence (D_{xy}) was calculated for each parent pair by summing the pairwise nucleotide distances between all MHC allele combinations between the parents. Note that some points have been moved slightly so as to not overlap entirely and the values on the *x* and *y* axes differ among figures **a**–**c**

in the two populations and no support for a survival advantage associated with nucleotide diversity during the fry stage in either population. In contrast to the results observed for the MHC class I, Quinsam larvae with low MHC class II nucleotide diversity were more likely to survive than expected by chance. Although our data provide support for balancing selection at the MHC class I locus in both populations of Chinook salmon, the data are mixed at the class II locus with evidence for directional selection in one of the populations. Thus, our data suggest a complex pattern of selection at the MHC across loci and populations.

Directional selection may favour a single allele at an MHC locus if the allele is closely linked to parasite resistance (Blanchet et al. 2009). During the larvae stage in the Quinsam population, we found evidence that offspring with lower MHC class II nucleotide divergence survived better than expected under our null hypothesis. We have previously reported that MHC class II genetic variation is dominated by a single allele in several Chinook salmon populations in British Columbia, including the Quinsam and Big Qualicum populations studied here (Evans et al. 2010; also see Miller et al. 1997; Neff et al. 2008). This low diversity may reflect directional selection for a “good gene” in these populations (Neff and Pitcher 2005; Pitcher and Neff 2006). Pitcher and Neff (2006) have reported evidence that a specific MHC class II allele is associated with a strong additive genetic effect on embryo and larvae survival in a Lake Ontario population of Chinook salmon. Moreover, in the guppy (*Poecilia reticulata*), populations are dominated by a single MHC class II allele that appears to provide resistance to a pervasive parasite that infects the fish (Fraser et al. 2010; Fraser and Neff 2010), and in a mammal, Meyer-Lucht and Sommer (2005) reported evidence of a significant advantage against nematode infections associated with a specific class II allele. Thus, unlike the MHC class I, directional selection may be common at the class II locus. In Chinook salmon, further study is needed to determine if there is an association between a specific pathogen and the MHC class II alleles that underlay the results we observed in the Quinsam population.

The strength and form of selection operating on juveniles may differ across life-history stages (Wedekind et al. 2008). In the Quinsam population, we found no evidence that survival was dependent on MHC class II diversity during the embryo stage, but during the larvae stage, individuals exhibited lower nucleotide divergence than expected assuming random survival. In contrast, in both the Quinsam and Big Qualicum populations, we found a selective advantage to MHC diversity at the class I during the embryo stage and potentially during the larvae stage. Wedekind et al. (2001, 2008) have similarly shown differences in the genetic architecture of whitefish (*Coregonus* sp.) survival as embryo development proceeds. These apparent locus-dependent differences in selection across life-history stages could be related to the onset of gene expression at the loci. The onset of MHC gene expression is currently unknown for most salmonids (but see Wedekind et al. 2004). However, a study of carp (*Cyprinus carpio*) has shown that MHC class II expression does not occur until larvae are 1 week old (Rodrigues et al. 1998).

For the MHC class I, gene expression in carp occurs much earlier and can be detected in embryos that are only 1 day old (Rodrigues et al. 1998). It is also possible that a shift in the strength of selection on the MHC across developmental stages could reflect exposure to different environmental conditions as development proceeds and hence a kind of gene-by-environment interaction (Wedekind et al. 2001). Thus, variation in gene expression during development or gene-by-environment interactions could explain the differential selection observed on the MHC loci.

The MHC is expected to evolve in response to the local pathogen community, and growing evidence suggests that fish parasite communities vary spatially (Arkoosh et al. 2004; Dionne et al. 2009; Evans and Neff 2009). We used a reciprocal translocation experiment to test for adaptive divergence in the MHC class I and II between our two study populations. During the larvae and fry stages, we found some evidence that control family replicates reared in their natal habitat were subjected to differing selection pressures than were translocation family replicates reared in a hatchery on the reciprocal river. Indeed, the translocation families showed no consistent selection on either MHC locus. These results provide compelling evidence for population-specific selection pressures on the MHC loci and adaptations to the local pathogen community. Bacterial pathogens appear to differ across the two populations, with the Big Qualicum population tending to exhibit higher bacterial infections and diversity than the Quinsam population (Evans and Neff 2009). Across Cyprinid fish species, Simkova et al. (2006) also found that parasite communities vary in diversity and that the diversity is tightly linked with MHC diversity exhibited among species. The authors argued that MHC diversity likely reflected variation in selection mediated by the local pathogen communities. If pathogen-based selection on the MHC in Chinook salmon is also driving the differences we observed at the MHC loci then our results suggest that such selection may vary over relatively small geographic scales.

In summary, this study provides evidence that genetic diversity at the MHC class I locus is linked to juvenile survival in Chinook salmon. Thus, the maintenance of MHC class I genetic diversity should be an important priority for conservation and management planning. We also found evidence of differential selection pressures on the MHC class II locus across populations, with evidence for directional selection in one population, but not in the other. In contrast to the class I locus, management plans that strive for genetic diversity at the MHC class II may in fact be detrimental to juvenile survival in some populations. Detailed understanding of selection pressures on the different MHC loci across populations is clearly warranted. Future research should focus on examining the sources of these differing forms of selection as such knowledge is critical for predicting and improving the freshwater survival of Chinook salmon juveniles.

Acknowledgments Many thanks to L. Clint and D. Ewart for permission to conduct this research in the Big Qualicum and Quinsam hatchery facilities. We also thank all of the staff at the Big Qualicum, Quinsam, and Rosewall hatcheries (Department of Fisheries and Oceans Canada, Pacific Region) for their significant logistical support throughout the duration of the experiment. Thanks to S. Garner for assistance setting up crosses, to V. Padmanabhan for assistance with the microsatellite genotyping, and to R. Hepburn and K. Ambacher for assistance with DNA extraction. A. Lachance provided access to the SSCP equipment. Eric Anderson and four anonymous reviewers provided helpful comments on the manuscript. Funding was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) PGS D Scholarship and an Ontario Graduate Scholarship in Science and Technology to MLE, NSERC Discovery/Accelerator grants and Canadian Foundation for Innovation/Ontario Innovation Trust grants to BDN, and NSERC Discovery and Canada Research Chair grants to DDH.

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