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MHC class IIB alleles contribute to both additive and nonadditive genetic effects on survival in Chinook salmon

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Abstract

The genes of the major histocompatibility complex (MHC) are found in all vertebrates and are an important component of individual fitness through their role in disease and pathogen resistance. These genes are among the most polymorphic in genomes and the mechanism that maintains the diversity has been actively debated with arguments for natural selection centering on either additive or nonadditive genetic effects. Here, we use a quantitative genetics breeding design to examine the genetic effects of MHC class IIB alleles on offspring survivorship in Chinook salmon (*Oncorhynchus tshawytscha*). We develop a novel genetic algorithm that can be used to assign values to specific alleles or genotypes. We use this genetic algorithm to show simultaneous additive and nonadditive effects of specific MHC class IIB alleles and genotypes on offspring survivorship. The additive effect supports the rare-allele hypothesis as a potential mechanism for maintaining genetic diversity at the MHC. However, contrary to the overdominance hypothesis, the nonadditive effect led to underdominance at one heterozygous genotype, which could instead reduce variability at the MHC. Our algorithm is an advancement over traditional animal models that only partition variance in fitness to additive and nonadditive genetic effects, but do not allocate these effects to specific alleles and genotypes. Additionally, we found evidence of nonrandom segregation during meiosis in females that promotes an MHC allele that is associated with higher survivorship. Such nonrandom segregation could further reduce variability at the MHC and may explain why Chinook salmon has one of the lowest levels of MHC diversity of all vertebrates.

Keywords: Chinook salmon, fitness, genetic quality, major histocompatibility complex, overdominance, rare allele

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Introduction

In vertebrates, the genes of the major histocompatibility complex (MHC; in humans referred to as human leucocyte antigen loci) are important candidates for fitness effects through their role in disease and pathogen resistance (Potts & Wakeland 1990; Apanius *et al*. 1997; Bernatchez & Landry 2003). The MHC genes code for proteins that present pathogens to the immune system. Specifically, MHC class I and class II genes encode cell-surface proteins capable of binding and presenting short peptides to T cells. In general, the MHC class I proteins present peptides derived from the cell cytoplasm to the T cells, whereas the class II proteins present exogenously derived peptides to T cells (Klein & Figueroa 1986; Klein 1990).

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In many populations, the MHC loci are highly polymorphic and most individuals are heterozygous (Bernatchez & Landry 2003). The mechanisms that maintain genetic diversity at the MHC have been actively debated and studies have described roles of both natural and sexual selection (Penn 2002; Bernatchez & Landry 2003; Neff & Pitcher 2005; Ziegler *et al*. 2005). The role of natural selection in maintaining MHC genetic diversity involves either nonadditive genetic effects leading to overdominance, in which case heterozygous individuals have increased survivorship because they are able to present a broader array of antigens and thereby resist a broader array of pathogens (Doherty & Zinkernagel 1975; Hughes & Nei 1988), or additive genetic effects leading to negative frequency dependent selection, in which case a coevolutionary arms race between specific MHC alleles and parasites results in the cycling in frequency of different alleles (rare-allele hypothesis; Clarke & Kirby 1966). The role of sexual selection in maintaining MHC genetic diversity can

parallel natural selection and may involve disassortative mating to ensure that offspring are heterozygous at the MHC and thus maximally resistant to disease (Penn *et al.* 2002; Milinski *et al*. 2005; Ziegler *et al*. 2005) or perhaps to ensure that parents are unrelated and thus to minimize inbreeding depression in the offspring (Potts & Wakeland 1993).

Several studies have performed experiments that challenge families of known MHC genotype with pathogens. First, Penn *et al*. (2002) found that MHC heterozygous mice (*Mus domesticus*) released into seminatural enclosures had greater survival, gained more weight and were better able to clear *Salmonella* and *Listeria* infection as compared to MHC homozygotes. Heterozygotes were more resistant than the average of two corresponding homozygotes, but they were not more resistant than both, indicating that the benefits accrued by MHC heterozygotes were due to resistance being dominant rather than overdominant. Second, Lohm *et al*. (2002) and Grimholt *et al*. (2003) investigated interactions between specific MHC alleles (class I and II) and resistance to various pathogens in Atlantic salmon (*Salmo salar*). In both studies, specific alleles were consistently associated with higher offspring survivorship, suggesting additive genetic variance for pathogen resistance in this species. Finally, Arkush *et al*. (2002) used *in vitro* fertilization techniques with Chinook salmon (*Oncorhynchus tshawytscha*) to produce individuals that were either homozygous or heterozygous at the MHC class II B gene. They then exposed the families to infectious haematopoietic necrosis virus (IHNV), the bacterium *Listonella anguillarum*, and *Myxobolus cerebralis*. There was no difference in survivorship among families when infected with either *Listonella anguillarum* or *Myxobolus cerebralis*. Conversely, individuals with one particular allele (*Onts wr3*) showed increased survivorship compared to individuals lacking this allele when infected with IHNV. IHNV causes epizootics in salmon and is considered to be one of the most important pathogens affecting these fishes (Wolf 1988). These studies suggest that additive genetic effects could lead to negative frequency dependent selection between MHC alleles and their associated pathogens. It is less clear whether or not nonadditive effects lead to overdominance and contribute to the maintenance of variation at the MHC (but see McClelland *et al*. 2003; for other challenge studies, see Kurtz *et al*. 2004; Mjaaland *et al*. 2005).

In this study, we use Chinook salmon and a novel genetic algorithm to partition genetic variance in offspring survival to additive and nonadditive genetic effects at the MHC class IIB locus. Chinook salmon are a Pacific salmon that breed in freshwater streams on the west coast of North America. They now have been introduced to other parts of North America, including the Great Lakes (Crawford 2001). Breeding begins when adults return to headwaters and females compete for nesting sites called redds (Healey 1991). Males also compete and form spawning hierarchies just downstream of receptive females. Typically, the largest males obtain positions at the front of the hierarchies and closest to the females. When females oviposit, males rush into the nest and fertilize the eggs. Females then remain until death to defend their nests from superimposition by other females. Conversely, males provide no care, but only sperm (i.e. genes) to the offspring.

Materials and methods

Parents and breeding

The Chinook salmon used in this study were collected using standard electroshock methods from a winter run in the Credit River, which flows into Lake Ontario. Chinook salmon have been stocked in Lake Ontario for about 36 years (Crawford 2001). On 6 October 2000, 11 males and 11 females were captured and eggs or milt were collected and placed in individual plastic bags. Care was taken to ensure that the gametes were not exposed to any water to prevent egg hardening or activation of the milt. The bags were stored at approximately 10 °C (the temperature of the river water) and transported back to the hatchery. A sample of tissue from the tail was also taken from each adult and preserved in 95% ethanol for genetic analyses.

We performed all 121 possible crosses of 11 males and 11 females in replicate (n = 242 families total). Eggs from each female were divided into 11 pairs of containers with 150 eggs each. Sperm concentration was determined for each male prior to the milt addition using a haemocytometer (methods in Pitcher *et al*. 2003), and the volume of milt was adjusted to ensure that equal numbers of sperm were introduced into each container. Egg samples were used in the same order as the females were collected and were timed to ensure that fertilization occurred about 90 min after collection. This fertilization protocol has been shown to result in fertilization rates in excess of 99% for salmon (Vronskiy 1972; Healey 1991). Next, each full-sib family was randomly allocated to a cell on a Heath incubation tray. Each tray had plexi-glass partitions that formed 16 equal sized cells. The Heath trays were then exposed to natural, untreated river water that ranged from 6 °C to 13 °C during the experiment.

Each day for 80 days postfertilization (about the end of the endogenous feeding stage), the trays were checked and the number of dead offspring within each full-sib family (cell) were numerated and removed. Although we did not assay the specific causes of mortality, common sources of offspring mortality in Ontario salmon hatcheries include water molds (*Saprolegnia* spp.), furunculosis (*Aeromonas salmonicida*), infectious pancreatic necrosis virus, and *Flavobacterium* diseases such as bacterial gill disease and cold-water disease (Bruneau *et al*. 1999). Overall survivorship was determined for each cell by dividing the number of surviving offspring by 150. Thus, for each family, we

had two measurements of survivorship. At the end of the experiment, five offspring from each cell (i.e. 10 from each full-sib family) were preserved in 95% ethanol.

Genetic analysis

DNA was extracted from either adult or offspring tissue using a simple proteinase K digestion (Neff *et al*. 2000). Using primers published in Docker & Heath (2002), we used polymerase chain reaction (PCR) to amplify 294 bases of the class IIB region of the major histocompatibility complex. These 294 bases encompass the variable part of the class II protein-binding region, which is responsible for binding foreign peptides and presenting them to T cells. Following Docker & Heath (2002), the PCR product from duplicate reactions were digested using either restriction enzymes *Rsa*I or *Dde*I. The restriction-enzyme-treated product was then run on a 2% agarose (*Rsa*I) or 4% high-resolution agarose (*Dde*I) gel (Sigma). This combination of enzymes allows the identification of the three major alleles in our population, which were confirmed by cloning and sequencing. Each of these alleles shows homology to previously published sequences in Chinook salmon comprising *Onts 1*, *Onts Ha71c*, and *Onts wr3* (Fig. 1; see Supplementary material for sequences). Hereafter we referred to these three alleles as 1, 2 and 3, respectively. We genotyped all of the adults and a subset of the offspring. The adult genotypes were tested against Hardy–Weinberg expected proportions using GENEPOP version 3.4 (Raymond & Rousset 1995).

To examine adult MHC genotypic correlates of offspring survival, we developed a genetic algorithm using the C++ computer programming language. The program partitions variance to both additive ($s_i + d_j$, i.e. sum of sire and dam additive effects) and nonadditive (I_{ij}) genetic effects by fitting familial data (in our case, mean survivorship) to the following general linear model (from Lynch & Walsh 1998, p. 598):

$$z_k = \mu + s_i + d_j + I_{ij} + e_k;$$

where z_k is the predicted mean phenotype of the kth family, μ is the mean phenotype across all families, and e_k is the deviation of the observed mean phenotype of the kth family from the model's prediction. Our program calculates the values of s_i, d_j and I_{ij} that minimized the sum of the squares of the deviations, e_k, across all families. The program utilizes the Downhill Simplex method (Nelder & Mead 1965) presented by Press *et al*. (1992). This routine tries numerous values of s_i, d_j and I_{ij} and, by evaluating both the value of $\sum e_k^2$ and its gradient, is able to efficiently search the plane and find where $\sum e_k^2$ reaches its minimum. Based on this best fit, the coefficient of determination (r^2) is also calculated (Zar 1999, p. 335).

To determine the significance of the model, the program employs a randomization routine. The routine randomizes the familial phenotypic data and re-calculates values of s_i, d_j and I_{ij}, and the coefficient of determination. The routine is repeated for a total of 1000 iterations and the resulting distributions are used to determine the significance of each value calculated from the original data. Provided the overall model is significant, no correction is required for multiple tests when interpreting the significance of individual terms because each term is not independent. We

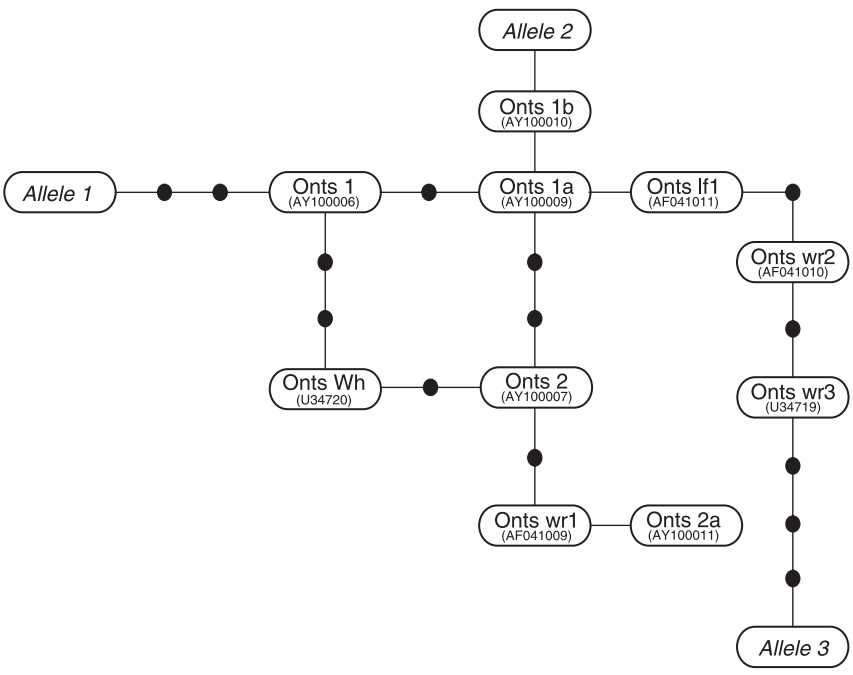

Fig. 1 Schematic diagram depicting nucleotide network for MHC class IIB sequences from Chinook salmon (*Oncorhynchus tshawytscha*). The filled circles between alleles denote the number of nucleotide substitutions or indels separating alleles and the lines denote possible evolutionary pathways relating the alleles. The three alleles from the current study are denoted by italic font. All other names refer to alleles cloned from other populations and recorded on GenBank under the corresponding accession number (in parentheses). Relationships are based on 196 bases (starting with the open reading frame) common to all of the sequences.

have made our algorithm available as an executable file downloadable at http://publish.uwo.ca/~bneff/links.htm.

To ensure that the model provided accurate (i.e. unbiased) estimates of additive and nonadditive genetic effects, we generated 1000 pseudo-data sets for each of three combinations of additive and nonadditive effects at a locus with three equally common alleles. The data were generated by randomly assigning parental genotypes for 100 families, determining the proportions of each offspring genotype (based on Mendelian inheritance), and calculating the associated survivorship based on the specified genetic effects. A random error was added to the survivorship value for each family to introduce 'noise' into the data. The genetic algorithm was then used to calculate the coefficient of determination and the additive and nonadditive genetic effects for each of the 1000 data sets of 100 families. This analysis revealed that the model provided accurate estimates. The data can be downloaded as a Microsoft Excel file from the link previously noted. In addition, this approach can be used by researchers to determine optimal experimental design (e.g. sample sizes) to ensure appropriate statistical power given a hypothesized effect size for each genetic effect.

For our MHC analysis, we used the additive and nonadditive effects determined from the genetic algorithm to calculate the survivorship value for each MHC genotype. Based on the allele frequencies calculated from the adult genotypes, we determined the net selection on each allele assuming random mating. This was accomplished by multiplying the normalized frequency of each of the three genotypes containing a focal allele by the survivorship value of that genotype and then summing over the three genotypes. To assess the variance associated with these estimates, we used a resampling routine. One thousand data sets were generated from the original family survivorship data by drawing families at random with replacement. These data sets were used to recalculate the MHC allele and genotype survivorship values and to assess the variance (reported as a standard deviation) in the net selection value for each allele. The resampled data sets were also used to determine the statistical probability that selection on each allele was negative, positive or neutral. Finally, we used the parental and offspring MHC data to test predictions of the genetic algorithm. We compared the numbers of each offspring genotype to expectations from two models: (i) null model, which assumes random segregation of alleles but no MHC-dependent survivorship; and (ii) MHC model, which assumes random segregation of alleles and MHC-dependent survivorship.

Results

There were a total of 36 300 eggs across the 242 families. At the termination of our experiment, survivorship among these families averaged 71 ± 19% (SD) (range = 13–99%; Table 1). Across family replicates, there was a high correlation in survivorship ($r = 0.92$, $P < 0.001$, $n = 121$). From the adult MHC genotypes we determined that the frequency of the three alleles in our population were 0.25 (allele 1), 0.20 (allele 2), and 0.55 (allele 3) and that the genotypes did not deviate from expected Hardy–Weinberg proportions (GENEPOP: $P = 0.93$). The genetic algorithm revealed both additive and nonadditive effects of adult MHC genotype on mean offspring survivorship (Table 2). For survivorship, the '2' allele was partially dominant to the '1' allele (because the 1/2 genotype survivorship value was greater than the mean of the 1/1 and 2/2 values and was closer to the 2/2 value), while the '1' allele was partially dominant to the '3' allele. The negative nonadditive effect of the 2/3 genotype led to underdominance, indicating that these alleles were 'incompatible genes.' These values appeared independent of the architecture of the remaining genome as omission of any one dam or sire had only minor effects on the values (Table 3).

Calculation of net selection on the three MHC class IIB alleles showed that the mean fitness (i.e. survivorship value) of the '1' allele was 0.64 ± 0.02 (SD), the '2' allele was 0.69 ± 0.03, and the '3' allele was 0.73 ± 0.01. Thus, assuming Hardy–Weinberg equilibrium and given the expected mean survivorship across all genotypes as 0.71, these survivorship values indicate negative selection on the '1' allele ($P < 0.001$), and suggest positive selection on the '3' allele ($P = 0.075$), but no net selection on the '2' allele ($P = 0.18$). The P values are based on the 1000 resampled data sets and denote the probability that the selection value is less than (alleles '1' and '2') or greater than (allele '3') the mean survivorship value.

The subset of offspring that we genotyped included all 10 offspring from families whose parental genotypes were expected to provide potential evidence of differential mortality based on MHC genotype (i.e. to yield offspring with genotypes associated with high survivorship and offspring with genotypes associated with low survivorship). Specifically, we selected crosses with one parent of genotype 1/3 and the other parent of genotype 3/3 (male 1/3: $n = 12$ families or 120 offspring; female 1/3: $n = 8$ families or 80 offspring) and with both parents of genotype 1/3 ($n = 8$ families or 80 offspring; see Supplementary material). From the male 1/3 by female 3/3 crosses, we found 51–1/3 offspring and 69–3/3 offspring, which is consistent with the MHC model (binomial $P = 0.32$), but unlikely based on the null model (binomial $P = 0.060$). From the female 1/3 by male 3/3 crosses, we found 29–1/3 offspring and 51–3/3 offspring, which is unlikely to occur based on either the MHC model (binomial $P = 0.074$) or the null model (binomial $P = 0.009$). For these latter crosses, there was an excess of 3/3 offspring relative to both model predictions. From the 1/3 by 1/3 crosses, we found 11–1/1, 33–1/3, and

Table 1 MHC class IIB genotypes for the parental Chinook salmon and the associated survivorship (%) of the 242 families. The two values for each parent pair represent the survivorship of the replicate families. Bold numbers at the base of columns or end of rows represent the mean survivorship (± 1 SE) for each male or female, respectively. Male and females are ordered in terms of mean offspring survival rank. Each parent's identification appears in parentheses where 'd' denotes dam and 's' denotes sire

Female	Male genotype	1 (sj) 2/3	2 (se) 1/3	3 (sh) 3/3	4 (si) 2/2	5 (sd) 1/3	6 (sc) 2/3	7 (sk) 1/3	8 (sf) 3/3	9 (sa) 3/3	10 (sg) 3/3	11 (sb) 1/3	Mean
1 (da)	3/3	97.3, 98.7	93.3, 93.3	94.0, 95.3	93.3, 98.0	86.7, 92.0	94.7, 96.0	86.7, 94.7	86.0, 94.7	90.7, 97.3	75.3, 83.3	85.3, 88.0	**91.6 ± 1.7**
2 (dj)	3/3	94.0, 95.3	91.3, 93.3	92.0, 95.3	94.0, 96.0	90.7, 92.7	87.3, 92.7	94.0, 94.0	96.0, 97.3	68.7, 74.7	87.3, 93.3	72.7, 74.0	**89.4 ± 4.8**
3 (db)	3/3	94.0, 96.0	88.7, 95.3	90.7, 94.0	86.7, 94.7	79.3, 93.3	88.0, 91.3	81.3, 88.0	84.7, 86.0	80.0, 81.3	82.7, 85.3	68.0, 78.0	**86.7 ± 2.1**
4 (dg)	2/3	90.7, 92.7	87.3, 94.0	58.0, 63.3	72.0, 92.7	82.0, 86.7	83.3, 90.7	79.3, 88.0	75.3, 76.0	86.0, 91.3	63.3, 76.0	80.7, 89.3	**81.8 ± 3.8**
5 (df)	1/1	76.7, 82.0	83.3, 86.7	78.0, 81.3	81.3, 82.0	79.3, 87.3	82.0, 83.3	73.3, 74.0	62.7, 83.3	68.7, 76.7	74.0, 74.0	66.7, 73.3	**77.7 ± 2.0**
6 (dd)	1/2	78.7, 88.0	80.0, 85.3	84.0, 85.3	86.0, 87.3	84.7, 90.0	74.7, 76.0	80.7, 86.7	70.0, 70.0	62.0, 78.0	67.3, 74.7	48.0, 48.7	**76.7 ± 3.6**
7 (de)	1/2	68.0, 74.7	76.7, 83.3	85.3, 88.0	64.7, 67.3	71.3, 73.3	76.7, 82.7	56.7, 58.0	70.7, 82.0	53.3, 77.3	48.7, 50.7	53.3, 54.7	**69.0 ± 3.7**
8 (di)	1/3	76.7, 79.3	58.7, 68.7	79.3, 80.0	68.7, 70.0	66.0, 70.7	55.3, 55.3	52.0, 66.7	68.0, 76.0	28.0, 32.0	46.0, 47.3	13.3, 34.7	**58.8 ± 5.3**
9 (dc)	1/3	55.3, 65.3	63.3, 66.7	62.7, 67.3	62.0, 67.3	72.7, 76.0	52.0, 61.3	53.3, 54.0	59.3, 74.7	39.3, 48.0	38.7, 50.7	40.0, 40.7	**57.8 ± 3.4**
10 (dh)	2/3	70.7, 72.0	56.7, 61.3	46.7, 57.3	46.7, 62.7	39.3, 46.0	56.0, 67.3	64.0, 66.0	26.7, 27.3	37.3, 47.3	33.3, 34.7	39.3, 43.3	**50.1 ± 4.4**
11 (dk)	1/2	46.7, 62.7	52.7, 64.7	55.3, 61.3	54.0, 61.3	54.0, 55.3	32.0, 46.0	41.3, 46.7	46.0, 58.7	23.3, 24.7	32.0, 36.7	20.7, 20.7	**45.3 ± 4.3**
	Mean	**79.8 ± 4.4**	**78.4 ± 4.3**	**77.0 ± 4.6**	**76.8 ± 4.6**	**75.9 ± 4.7**	**73.8 ± 5.4**	**71.8 ± 5.0**	**71.4 ± 5.8**	**62.1 ± 7.1**	**61.6 ± 6.1**	**56.1 ± 6.9**	

Table 2 Additive and nonadditive genetic effects of MHC class IIB alleles on survivorship in Chinook salmon. The results include the r^2, constant (μ), additive and nonadditive effects estimated using our genetic algorithm (left half of table). These values were used to calculate the expected survivorship values for each possible genotype (right half of table)

Model output	Survivorship (%)	Genotype	Survivorship value (%)
r^2	0.09*	—	—
Constant	0.71	—	—
Additive			
1	–0.06**	11	0.59
2	0.00^{NS}	22	0.71
3	0.06*	33	0.83
Nonadditive			
1 + 2	0.03^{NS}	12	0.68
1 + 3	-0.07^{NS}	13	0.64
2 + 3	–0.09**	23	0.68

*$P < 0.05$; **$P < 0.10$; NS: $P \geq 0.10$.

36–3/3 offspring, which is unlikely to occur under either the MHC model or the null model (χ^2, $P < 0.01$ for both). We additionally examined two crosses with a female 1/3 by male 2/2. We selected these two crosses to assess if 1/3 females were overproducing the '3' haplotype in the secondary oocyte during oogenesis. We found no evidence of such a bias as 10 of each of the 1/2 and 2/3 genotypes were present in the 20 offspring. Omission of the two 1/3 females did not affect the basic pattern of the additive and nonadditive genetic effects (albeit the nonadditive effect of the 2/3 genotype doubled and was highly significant; see Table 3).

Discussion

We used a fully crossed quantitative genetic breeding design and a novel genetic algorithm to partition variance in offspring survival of Chinook salmon to additive and nonadditive genetic effects at the MHC class IIB locus. This algorithm is an advancement over traditional animal models that only partition variance in fitness to additive and nonadditive genetic effects, but do not allocate these effects to specific alleles and genotypes. Our analysis using this algorithm suggests that negative frequency dependent selection, but not overdominance, could contribute to polymorphism at this locus. For example, we found a positive additive effect of the '3' allele in our population that led to the 3/3 genotype having the highest survivorship value of all genotypes. In another study on the same species, a close homologue (*Onts wr3*) of this allele was associated with increased resistance to infectious haematopoietic necrosis virus (Arkush *et al*. 2002). The high prevalence of IHNV in many Chinook salmon populations (Wolf 1988)

Table 3 Additive and nonadditive genetic effects of MHC class IIB alleles on survivorship in Chinook salmon with one or two dams or one sire omitted from the analysis. The results include the r^2, constant (μ), additive and nonadditive effects for each allele or genotype estimated using our genetic algorithm

			Additive effects			Nonadditive effects		
Omitted parent	r^2	Constant	1	2	3	1 + 2	1 + 3	2 + 3
Dams								
da	0.05**	0.69	–0.04^{NS}	0.02^{NS}	0.05**	0.03^{NS}	–0.06^{NS}	–0.11***
db	0.06**	0.70	–0.05^{NS}	0.02^{NS}	0.06**	0.03^{NS}	–0.07^{NS}	–0.12***
dc	0.10**	0.73	–0.07^{NS}	0.00^{NS}	0.06**	0.03^{NS}	–0.05^{NS}	–0.11***
dd	0.11**	0.71	–0.07***	–0.02^{NS}	0.07**	0.04^{NS}	–0.07^{NS}	–0.08^{NS}
de	0.09**	0.72	–0.06^{NS}	0.00^{NS}	0.06**	0.03^{NS}	–0.08^{NS}	–0.10^{NS}
df	0.15**	0.71	–0.14**	0.01^{NS}	0.07**	0.07^{NS}	–0.07^{NS}	–0.07^{NS}
dg	0.09**	0.70	–0.05^{NS}	–0.03^{NS}	0.06**	0.06^{NS}	–0.07^{NS}	–0.07^{NS}
dh	0.17**	0.73	–0.08***	0.01^{NS}	0.07**	0.03^{NS}	–0.12***	–0.03^{NS}
di	0.10**	0.73	–0.05^{NS}	0.01^{NS}	0.06**	–0.02^{NS}	–0.06^{NS}	–0.13**
dj	0.06**	0.70	–0.05^{NS}	0.02^{NS}	0.06**	0.03^{NS}	–0.07^{NS}	–0.11***
dk	0.05**	0.74	–0.06^{NS}	0.02^{NS}	0.04**	0.03^{NS}	–0.04^{NS}	–0.05^{NS}
dc, di*	0.13**	0.74	–0.04^{NS}	0.00^{NS}	0.07**	–0.05^{NS}	–0.04^{NS}	–0.18**
Sires								
sa	0.10**	0.72	–0.09**	0.00^{NS}	0.06**	0.04^{NS}	–0.03^{NS}	–0.08^{NS}
sb	0.07**	0.73	0.02^{NS}	–0.01^{NS}	0.05**	–0.01^{NS}	–0.16**	–0.07^{NS}
sc	0.08**	0.71	–0.06^{NS}	0.02^{NS}	0.06**	0.03^{NS}	–0.06^{NS}	–0.11^{NS}
sd	0.10**	0.71	–0.11**	0.00^{NS}	0.06**	0.10^{NS}	–0.04^{NS}	–0.07^{NS}
se	0.10**	0.71	–0.10***	0.01^{NS}	0.06**	0.07^{NS}	–0.05^{NS}	–0.08^{NS}
sf	0.08**	0.71	–0.06***	0.00^{NS}	0.06**	0.03^{NS}	–0.08^{NS}	–0.06^{NS}
sg	0.10**	0.72	–0.08***	–0.01^{NS}	0.06**	0.04^{NS}	–0.04^{NS}	–0.05^{NS}
sh	0.08**	0.71	–0.04^{NS}	0.00^{NS}	0.06**	0.03^{NS}	–0.12^{NS}	–0.08^{NS}
si	0.12**	0.71	–0.05^{NS}	0.10^{NS}	0.07**	–0.09^{NS}	–0.06^{NS}	–0.30**
sj	0.08**	0.70	–0.06^{NS}	0.00^{NS}	0.06**	0.04^{NS}	–0.06^{NS}	–0.11***
sk	0.08**	0.71	–0.05^{NS}	0.00^{NS}	0.06**	0.02^{NS}	–0.10^{NS}	–0.08^{NS}

**$P < 0.05$; ***$P < 0.10$; NS: $P \geq 0.10$; *dams dc and di were both genotype 1/3.

may explain why *Onts wr3* and its close homologues are present in all populations examined to date (Miller *et al*. 1997; Heath *et al*. in press). In Atlantic salmon, two other studies have found associations between resistance to a specific pathogen and individual MHC alleles (Lohm *et al*. 2002; Grimholt *et al*. 2003) and additive genetic effects have been shown in several other fishes (e.g. Wedekind *et al*. 2001). Conceivably, as the prevalence of these various pathogens change in the environment, so will the survivorship value of the associated alleles, which could lead to negative frequency dependent selection and the fluctuation of allele frequencies through evolutionary time.

We also found a negative nonadditive effect of the 2/3 genotype on survivorship. Such negative effects have been reported in Atlantic salmon (Grimholt *et al*. 2003), although the authors of that study were unable to test for nonadditive effects (in part because our genetic algorithm was not previously available). In our case, the nonadditive effect led to underdominance (i.e. the 2/3 genotype had a lower survivorship value than either the 2/2 or 3/3 genotype), which could actually reduce polymorphism at the MHC class IIB locus. Such underdominance effects could have contributed to the erosion of MHC polymorphism in Chinook salmon and may explain why this species has one of the lowest levels of MHC diversity of all vertebrates (Miller *et al*. 1997; Heath *et al*. in press). There was no apparent nonadditive effect at any of the other heterozygous genotypes. Thus, it will be important for future studies to differentiate between specific heterozygotes when examining nonadditive effects as a possible mechanism that increases (or decreases) genetic diversity at the MHC (for further discussion, see Bernatchez & Landry 2003). Our genetic algorithm should facilitate such analyses.

The analysis of offspring MHC genotypes confirmed some of the predictions of our genetic algorithm. For example, the offspring genotype numbers from crosses between 1/3 males and 3/3 females were consistent with the MHC-dependent survivorship model, but inconsistent with the null model of random segregation and no MHC-dependent survivorship. The offspring genotype numbers from crosses between 3/3 males and 1/3 females and the 1/3 males and 1/3 females both revealed an excess of the

maternally inherited '3' allele. Interestingly, because the second polar body is not discarded until after fertilization (Bond 1996, p. 500), a crossover event (involving the MHC locus) earlier in oogenesis would lead to a secondary oocyte of genotype 1/3. When such an oocyte is fertilized by sperm containing the '3' allele, it could preferentially discard the 1 allele in the second polar body (see Wedekind *et al*. 1996 for a similar example in mice). Based on the two types of crosses for which a 3/3 bias was detected, we determined that the segregation bias was between 8% and 27%, which could increase a female's fitness by as much as 7%. We could rule out that 1/3 females were instead overproducing secondary oocytes with the '3' allele, for example by preferentially discarding the '1' allele in the first polar body, because the crosses between 1/3 females and the 2/2 male revealed no excess of the '3' haplotype. Interestingly, based on our MHC model, the 1/2 and 2/3 genotypes had equal survivorship values and so any bias in these latter crosses would not increase familial survivorship. Nevertheless, without examining the genotypes of secondary oocytes prior to fertilization or offspring that died, we cannot conclusively determine the actual source of the observed genotype bias (see Wedekind *et al*. 2004).

Our analysis revealed MHC class IIB allele- and genotype-dependent survivorship, and therefore suggests that this gene plays an important role in immunity during early development in Chinook salmon. The ontogeny of the immune system has been studied in several fishes (reviewed by Tatner 1996). For example, in Atlantic salmon, the development of lymphoid organs and the appearance of lymphocytes, both precursors to an MHC immune response, are present as early as 22 days prior to hatching (Ellis 1977). In sea bream (*Sparus aurata*), however, these organs and cells first appear as late as 47 days after hatching (Josefsson & Tatner 1993). Two other studies have directly examined expression of MHC genes in fish. Rodrigues *et al*. (1998) found that in carp (*Cyprinus carpio*) that MHC class I and II genes were transcribed 1 day postfertilization and 1 day posthatching, respectively, whereas Fischer *et al*. (2005) found that in rainbow trout (*Oncorhynchus mykiss*) the alpha unit of the class I was not expressed until 3 days posthatching. There are no data yet available for Chinook salmon, but these other studies indicate that the MHC class IIB is expressed about the time of hatching and possibly considerably earlier in development.

Estimates of additive genetic variance may in some cases be overestimated if environmental effects are correlated with certain genotypes (see Kotiaho *et al*. 2003). For example, if females with a certain genotype are healthier because of positive additive genetic effects and are able to produce larger eggs than females with a different genotype, than any observed effect on fitness will not only be due to maternal additive genetic effects but also maternal environmental effects. In the study by Kotiaho *et al*. (2003), unaccounted for maternal effects were estimated to lead to an inflation of 10–20% for the additive genetic effects. In our study, the '3' allele showed additive genetic effects and females with 3/3 had the highest offspring survivorship. Thus, it is conceivable that correlated maternal environment and additive genetic effects has led to an inflation of the additive genetic effects. However, the 3/3 females did not produce larger eggs (a measure of egg quality; Heath *et al*. 1999) than the other females, there was no significant relationship between mean egg diameter and family survivorship, and an analysis that removed the potential maternal environmental effects associated with egg diameter using residuals from a linear regression provided similar results (data not shown). Regardless, Kotiaho *et al*. (2003) rightly point out that associations between maternal environmental effects and additive genetic effects can lead to inflated estimates of the latter effect. This may be particularly problematic in systems that have extended maternal investment in offspring, such as species with maternal offspring provisioning. Fish mating systems with external fertilization, such as the Chinook salmon studied here, may provide ideal systems to examine the genetic effects of the MHC (or any other fitness locus) because there is no offspring provisioning outside of the egg yolk, and maternal environmental effects associated with egg yolk can be easily estimated by egg size or by other methods (Heath *et al*. 1999).

In conclusion, we found both additive and nonadditive genetic effects of MHC class IIB alleles on offspring survivorship in Chinook salmon. The additive effect supports the rare-allele hypothesis as a potential mechanism for maintaining genetic diversity at the MHC, but the nonadditive effect led to underdominance, which is inconsistent with the overdominance hypothesis and could instead reduce variability at the MHC. Our genetic algorithm will allow researchers to partition genetic effects on phenotype to specific alleles at candidate loci, and we hope will provide a better understanding of the genetic architecture of phenotypes and ultimately fitness.

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Supplementary material

The supplementary material is available from
http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2942/MEC2942sm.htm

Appendix 1 DNA sequences for the three MHC class IIB alleles found in our adult population of Chinook salmon. The numbers in parentheses denote the homology to previously published sequences in Chinook salmon (see Miller & Withler 1996; Kim *et al*. 1999; Arkush *et al*. 2002; Docker & Heath 2002). Any difference between the two sequences is noted with a slash (the first base represents our sequence and the second base represents the previously published sequence). Our sequences have GenBank accession numbers DQ450873–5.

Appendix 2 Offspring MHC class IIB genotype for selected families in Chinook salmon. Expected numbers of each genotype are given for the null model, which assumes random segregation of alleles, but no MHC-dependent survivorship, and for the MHC model, which assumes random segregation and MHC-dependent survivorship as determined from this study. The statistical fit to these models is provided based on either a binomial or χ^2 test.

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