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Major histocompatibility complex heterozygote advantage and widespread bacterial infections in populations of Chinook salmon (*Oncorhynchus tshawytscha*)

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Abstract

Despite growing evidence for parasite-mediated selection on the vertebrate major histocompatibility complex (MHC), little is known about variation in the bacterial parasite community within and among host populations or its influence on MHC evolution. In this study, we characterize variation in the parasitic bacterial community associated with Chinook salmon (*Oncorhynchus tshawytscha*) fry in five populations in British Columbia (BC), Canada across 2 years, and examine whether bacterial infections are a potential source of selection on the MHC. We found an unprecedented diversity of bacteria infecting fry with a total of 55 unique bacteria identified. Bacterial infection rates varied from 9% to 29% among populations and there was a significant isolation by distance relationship in bacterial community phylogenetic similarity across the populations. Spatial variation in the frequency of infections and in the phylogenetic similarity of bacterial communities may result in differential parasite-mediated selection at the MHC across populations. Across all populations, we found evidence of a heterozygote advantage at the MHC class II, which may be a source of balancing selection on this locus. Interestingly, a co-inertia analysis indicated only susceptibility associations between a few of the MHC class I and II alleles and specific bacterial parasites; there was no evidence that any of the alleles provided resistance to the bacteria. Our results reveal a complex bacterial community infecting populations of a fish and underscore the importance of considering the role of multiple pathogens in the evolution of host adaptations.

Keywords: bacteria, Chinook salmon, co-evolution, heterozygosity, local adaptation, major histocompatibility complex, spatial variation

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Parasitic organisms play an important role in the survival and reproductive success of their hosts (Anderson & May 1982; Kennedy 1985). Hosts may evolve in response to parasites, and variation in parasite communities across the landscape can drive local adaptation and genetic variation within host populations (Holmes 1983; Kennedy 1985; Summers *et al.* 2003). In vertebrates, the genes of the major histocompatibility complex (MHC) encode proteins that recognize and present pathogen-derived peptides to T-cells, which then mount a specific immune response against the pathogen (Klein 1986; Matsumura *et al.* 1992). Thus, the MHC provides an excellent opportunity to examine relationships between host and parasites because MHC genes are expected to evolve in response to variation in the parasite community (see Apanius *et al.* 1997; Bernatchez & Landry 2003; Sommer 2005; Piertney & Oliver 2006 for a review).

Perhaps one of the best examinations of MHC evolution in response to parasites is that of humans and malaria (Hill *et al.* 1991, 1994). Research has shown that

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particular MHC (or human leucocyte antigen) alleles confer resistance to malaria and that these alleles are found more frequently in geographic regions that are subject to intense levels of malarial transmission. These data suggest that malarial parasites and human MHC alleles exhibit a tightly linked evolutionary history (Hill *et al.* 1991, 1994). In addition, specific MHC alleles in birds, fishes and other mammals have been shown to be associated with disease resistance (e.g. Kean *et al.* 1994; Grimholt *et al.* 2003; Miller *et al.* 2004; Harf & Sommer 2005; Johnson *et al.* 2008; Fraser & Neff 2009). Thus, it is likely that the MHC has evolved in response to local pathogen communities in many species across the vertebrate taxa.

The MHC is one of the most polymorphic genetic regions described to date (Hedrick 1994; Hughes & Hughes 1995). The high degree of variation at the MHC is thought to be driven by some form of balancing selection such as heterozygote advantage, negative frequency-dependent selection, or fluctuating directional selection via spatial or temporal variation in the pathogen community within or among populations (reviewed by Apanius *et al.* 1997; Hedrick 1999, 2002; Bernatchez & Landry 2003; Sommer 2005; Piertney & Oliver 2006). Heterozygote advantage results when heterozygous individuals are better able to recognize and mount an immune response against a larger array of pathogens than homozygous individuals (Doherty & Zinkernagel 1975; Hughes & Nei 1988, 1989; e.g. Hedrick *et al.* 2001; Arkush *et al.* 2002; McClelland *et al.* 2003). Several studies have found that MHC class II heterozygotes exhibit lower parasite load (Hedrick *et al.* 2001; Froeschke & Sommer 2005; see Wegner *et al.* 2004 for a review) or have found an excess of heterozygotes within populations, which suggests selection for heterozygotes (Seddon & Baverstock 1999; van Haeringen *et al.* 1999; Peters & Turner 2008; Oliver *et al.* 2009; see Apanius *et al.* 1997; Garrigan & Hedrick 2003 for reviews). Negative-frequency dependent selection can also maintain high levels of genetic variation at the MHC when rare alleles confer higher fitness against pathogens in the environment when compared with more common alleles (Takahata & Nei 1990). Finally, spatial and temporal variation in the parasite community can maintain genetic variation when pathogen communities fluctuate in space or time and thereby change the selective pressure on the MHC (Hedrick 2002).

Despite the importance of understanding the evolutionary relationships between hosts and their parasites, most research has explored a limited set of community interactions – that being between a host and a single macroparasite (see Wegner *et al.* 2004; Collinge & Ray 2006). A few studies have documented multiple parasites within hosts, but these studies have largely focused on mammals (e.g. Penn *et al.* 2002; McClelland *et al.* 2003; Deter *et al.* 2008; Tollenaere *et al.* 2008), with only a few studies on fish (deSousa & Sila-Sousa 2001; Wegner *et al.* 2003; Dionne *et al.* 2007, 2009; Simkova *et al.* 2008). Thus, relationships between MHC adaptations of hosts to parasite communities in marine and aquatic ecosystems remain relatively understudied, even for species of significant economic importance. Moreover, in fishes, surveys for parasites have typically focused on examining the prevalence of specific helminth, arthropod, Protozoa or Myxozoa species (Marcogliese 2002; Wegner *et al.* 2003; Simkova *et al.* 2008). However, hosts in wild populations are vulnerable to infections from a complex community of parasites, which is likely to include many species of bacteria or viruses, and, as such, surveys for individual parasites may miss important sources of selection pressure on hosts (Dobson & Foufopolous 2001; Penn *et al.* 2002; McClelland *et al.* 2003; see Wegner *et al.* 2004 for a review). Thus, to gain a better understanding of the evolutionary importance of host–parasite interactions, more studies are needed to examine the diversity and structure of the parasite community affecting hosts in natural populations.

In this study, we characterize the parasitic bacterial community isolated from the kidney tissues of Chinook salmon (*Oncorhynchus tshawytscha*) fry. Internal organs are generally assumed to be sterile, so the isolation of bacteria from kidney tissue is taken as evidence of a bacterial species parasitizing the host (Atlas & Bartha 1998; deSousa & Sila-Sousa 2001). We use a 16s rDNA sequencing-based approach to identify and examine the prevalence of bacteria infecting the fry and compare the bacterial communities to MHC genetic variation at the class I-A1 and class II-B1 loci of the host. Chinook salmon are anadromous, semelparous breeders and adults typically return to their natal freshwater stream to breed after a period of 2–6 years maturing in salt water. Fry develop within discrete freshwater river systems for up to 1 year and thus may be subject to unique pathogen communities for a significant portion of their development. Moreover, fewer than 30% of young survive to migrate to the ocean, indicating that substantial mortality occurs during this life-history stage (Healy 1991).

To examine spatial and temporal variation in the parasitic bacterial community, we studied infection rate, diversity of bacteria, and bacterial community similarity within and among five populations of Chinook salmon fry in British Columbia, Canada across 2 years. To examine the potential influence of spatial and temporal variation in the bacterial community on MHC genes, we examined correlations between bacterial community similarity and population genetic divergence at MHC

genes. We also use our data to investigate the potential for MHC heterozygote advantage against bacterial parasite infections and incorporate a multivariate co-inertia analysis (Dolédec & Chessel 1994) to examine MHC allele–bacteria associations as potential evidence of negative-frequency dependent selection on MHC genes.

Methods

Fish sampling

In 2006 and 2007, between April 20 and May 20, we collected Chinook salmon fry from the Quinsam, Puntledge and Big Qualicum rivers located on the east coast of Vancouver Island (Fig. 1). We also collected samples between September 15 and October 1 in both years from the Kitsumkalum River (a tributary of the Skeena River) in northwestern British Columbia, and during the same period in 2007, from the main stem of the lower Skeena River, which is approximately 40 km west of the confluence of the Skeena and Kitsumkalum rivers (Fig. 1). Fry from the Kitsumkalum and lower Skeena were sampled later in the year because populations in the north exhibit slower growth than those on Vancouver Island. Fry were captured using baited gee traps, live traps and beach seining. The number of fry collected from each population is shown in Table 1. Individuals were transported to the laboratory where they were anaesthetized with an overdose of the anaesthetic MS-222 (Tricaine Methanesulfonate; Argent labs) and subsequently stored on ice until the dissections were conducted.

Following Thoesen (1994), we used aseptic techniques to dissect each fish and to collect a sample of the kidney tissue. The dissections began by disinfecting the body surface of each fish using 95% ethanol. Kidney tissue was then obtained and streaked onto tryptic soy agar (TSA; Becton-Dickson Inc) and *Cytophaga* agar plates (Thoesen 1994). Both types of media are well-established in the growth of bacterial fish parasites (Thoesen 1994). Care was taken during dissection to avoid contact with internal organs other than the kidney. Plates were incubated for up to 3 weeks at 20 °C in aerobic conditions, and were initially examined for the presence of bacterial colony growth 48 h after plating. When a colony was observed, a small section was collected and re-streaked onto a new plate to assure purity of the bacteria sample. When more than one morphologically distinct bacterial colony was isolated, we separately re-streaked these colonies onto new plates.

Bacterial identification

DNA from bacterial samples was obtained by suspending a small portion of a colony in 10% chelex 100 resin (Sigma-Aldrich Canada Ltd.). Samples were vortexed in the chelex slurry for 20 s, incubated at 95 °C for 20 min and then vortexed for a final 20 s. The samples were then centrifuged, leaving the DNA dissolved in the supernatant. We used PCR to amplify a portion of the

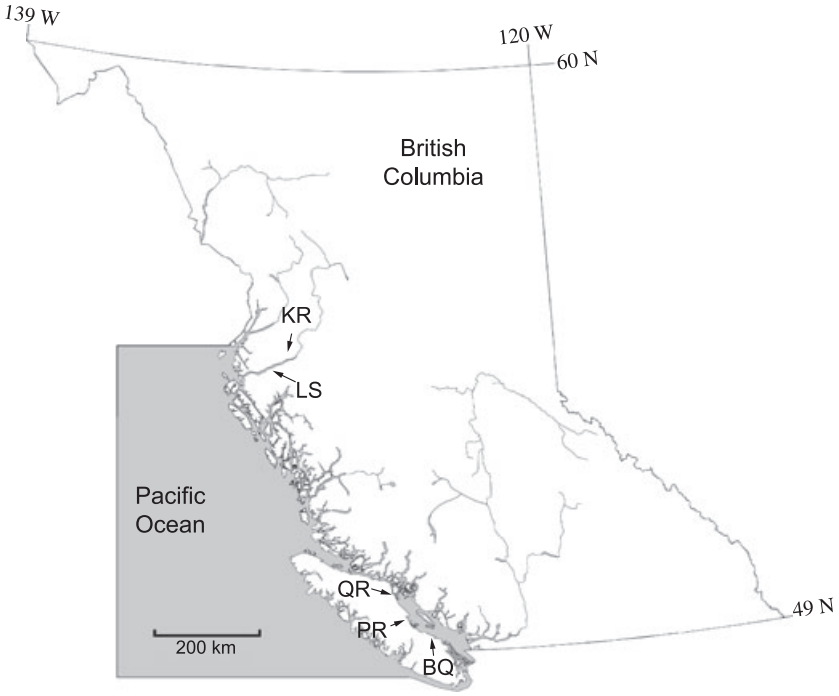

Fig. 1 Map of British Columbia indicating the locations of the five populations of Chinook salmon (*Oncorhynchus tshawytscha*). Populations of Chinook salmon comprise: KR, Kitsumkalum River; LS, lower Skeena River; QR, Quinsam River; PR, Puntledge River; BQ, Big Qualicum River. Figure modified from Heath *et al*. (2006).

Table 1 Prevalence of bacteria isolated from the kidney tissue of Chinook salmon (*Oncorhynchus tshawytscha*)

Population	*N*	N_C	Affiliation (Genus/Species)	GenBank accession	% Match	% Prevalence
Big Qualicum River	90	28	***Bacillus* sp.**	EU218867	96	1
			***Arthrobacter* sp.**	DQ173023	96	2
			Bacillaceae BL-106	EQ596921	95	1
			Carnobacterium divergens	EU621983	96	1
			Lysobacter brunescens	AB161360	95	1
			Methylobacterium sp.	FJ267581	97	1
			Moraxella osloensis	AM293373	97	3
			***Mycobacteria* sp.**	EF451723	96	3
			***Pseudomonas* sp.**	EU239204	98	1
			Pseudomonas putida	EU627168	97	1
			***Roseomonas* sp.**	AY167826	98	2
			Streptomyces chryseus	EU593575	99	1
			***Streptomyces* sp. M045**	AY644669	97	1
			***Streptomyces* sp.**	AB124379	97	1
			Comamonadaceae	EF370557	94	1
			***Corynebacterium* sp. 3LF25TD**	EU417660	97	2
			Friedmaniella sp.	AF409005	99	1
			Schineria sp. CHNDP40	DQ337535	95	1
			Sphingomonas sp.	AM900788	99	1
			Sphigomonas sp.	AY167833	96	1
			Yersinia ruckeri	AF366385	98	1
		2	No significant similarity			
Quinsam River	106	20	*Arthrobacter* sp. ISSDS-854	EF634027	91	1
			Acidovorax sp.	FJ193989	96	1
			***Bacillus* sp.**	AY741506	98	1
			Curtobacterium sp.	EU236753	95	1
			Microbacterium sp.	FJ267583	97	1
			Microbacterium sp.	EF540512	96	1
			Microbacterium sp.	AB042073	93	1
			Microbacterium hatanonis	AB274908	96	1
			Mycobacterium komossense	AY438077	95	1
			***Erwinia* sp. CYEB-26**	FJ422383	95	1
			***Pseudomonas* sp.**	AY653220	97	2
			***Pseudomonas* sp.**	FM161424	97	1
			Sphingomonas sp.	DQ512745	97	1
			***Rahnella* sp.**	AM419020	96	1
			Rhizobium sp.	EU781656	96	1
			***Serratia* sp. BS18**	EU031768	99	1
			***Staphylococcus* sp. AM14**	AJ971853	97	1
			***Streptomyces* sp. M060706-9**	EU589408	95	1
			Uncultured bacteria	FM873011	94	1
		7	No significant similarity			
Puntledge River	91	7	***Staphylococcus* sp. AM14**	AJ971853	99	1
			***Streptomyces* sp. 919**	EU159564	97	1
			***Acinetobacter* sp.**	EU705479	91	1
			Acinetobacter junii	AJ786647	92	1
			Comamonadaceae P1D3	AM482163	98	1
			***Corynebacterium* sp. 3LF25TD**	EU417660	92	1
			Uncultured actinobacterium	DQ828433	96	1
		1	No significant similarity			
Kitsumklaum River	60	7	***Erwinia* sp. CYEB-26**	FJ422383	85	2
			***Pseudomonas* sp. SPW1**	EU391589	98	2
			***Pseudomonas* sp. XH1**	FJ424509	98	2

Table 1 *Continued*

Population	*N*	N_C	Affiliation (Genus/Species)	GenBank accession	% Match	% Prevalence
Pseudomonas fluorescens	AY581137	99	2			
Streptomyces olivochromogenes	EU570547	95	2			
Pedobacter sp.	AY599665	96	2			
			Actinobacterium RG-9.	AY561575	94	2
		2	No significant similarity			
Lower Skeena River	21	5	***Hafnia* sp.**	EU159563	92	5
			Alpha proteobacterium	AY642568	92	5
			Kocuria sp.	FJ155338	95	5
			Yersinia kristensenii	EU434503	97	10
		1	No significant similarity			

Samples were obtained from fry collected from five populations in British Columbia, Canada. Data comprise number of fish collected from each population (*N*), number of bacterial colonies isolated (N_C), closest bacterial affiliation, GenBank accession number for the closest sequence matching the isolated bacteria, the associated per cent match between the sequences, and the prevalence of the bacteria (per cent of individuals in each population with bacterial infection). Bacterial genera that have been implicated as fish pathogens based on Austin & Austin (2007) are noted in bold.

bacterial 16s rDNA using a previously described primer pair (ULF500: CCTAACACATGCAAGTCGA and ULR500: CGTATTACCGCGGCTGCTGG; Avaniss-Aghajani *et al.* 1996). The primer pair amplifies approximately 500 bp of the 16s rDNA gene corresponding to the region between 46 and 537 bases in *Escherichia coli*. PCR reactions were performed in 50 μL volumes composed of 0.1 mM of each dNTP (Invitrogen, California, USA), 0.05 U/μL *Taq* (Invitrogen), 1× reaction buffer, 2.5 mM $MgCl_2$ and 0.1 mM of each primer. We used USP-grade water (Sigma-Aldrich Canada Ltd.) in the PCR reactions and a negative control was run during each PCR reaction to confirm that PCR reagents were free from DNA contamination. The PCR cycle consisted of an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 30 s. The last cycle was followed by a final extension step at 72 °C for 5 min. We used direct sequencing of the 16s rDNA amplicon to identify bacteria.

Bacteria were identified by aligning the DNA sequence to the GenBank database using BLASTN (basic local alignment search tool; http://www.ncbi.nlm.nih.gov/BLAST; Altschul *et al.* 1990). We used MEGA to construct a neighbour-joining phylogenetic tree for each population, employing the Jukes-Cantor correction, to provide further support for our classification of bacteria isolated as unique phylotypes. Herein we use the term 'phylotype' to describe each bacterium that aligned to a unique GenBank sequence, as most bacteria were not identified to a described species. Bootstrap support for phylogenetic tree branches was obtained over 500 repetitions.

Bacterial community variation

For each of the five Chinook salmon populations, we calculated the per cent infection rate (number of individuals with bacterial infections/total number of individuals sampled within a population), the bacterial diversity (mean number of bacterial phylotypes per individual within each population) and the per cent prevalence of each bacterial phylotype (number of individuals with a particular bacterial infection/total number of individuals sampled within a population). To examine differences in the occurrence of bacterial infections and bacterial diversity across years and populations, we employed a generalized linear model (GLM) in SPSS v. 17. To examine variation in infection rate, a binomial distribution was used in the GLM, with infection status as the response variable and population and year as fixed factors in the model. To examine variation in bacterial diversity, a GLM with a Poisson distribution was used, with number of bacteria per individual as the response variable and year and population as fixed factors in the model. Pairwise comparisons of bacterial infection rates and bacterial diversity were run within the GLM to determine the significance of the differences among pairs of populations. We used the UniFrac metric of phylogenetic community similarity to examine bacterial community similarity among populations and between years within populations (Lozupone *et al.* 2006). The UniFrac metric measures the divergence between different populations as the fraction of the branch lengths found in the phylogenetic tree that are unique within each population. A neighbour-joining phylogenetic tree (applying the Jukes-Cantor correction)

for all bacteria identified in all populations was constructed in MEGA (Molecular Evolutionary Genetics Analysis) software v.4 (http://megasoftware.net, Tamura *et al.* 2007) using *Haloterrigena thermotolerans* strain SYS as an outgroup. *Haloterrigena thermotolerans*, an archaen species, was utilized as an outgroup because of the broad taxonomic distribution of bacterial phylotypes identified in our study (see Ciccarelli *et al.* 2006). We examined bacterial community similarity among populations by calculating pairwise population estimates of the weighted UniFrac metric. We examined temporal variation in the bacterial community by conducting pairwise comparisons between the bacterial community found in each population (Big Qualicum, Quinsam, and Kitsumkalum only) in each year using weighted UniFrac estimates. UniFrac metric values range from 0 to 1, with 0 indicating that the communities under comparison exhibit 100% similarity in their phylogenetic structure, and 1 indicating that the communities are highly differentiated. The significance of the pairwise estimates of community similarity was determined using the program's default 100 permutations and Bonferroni correction for multiple comparisons. We examined isolation by distance in the UniFrac metric estimates of bacterial community similarity in GENEPOP v.3.4 (Raymond & Rousset 1995). Significance of the isolation by distance relationship was determined using a Mantel test conducted over 1000 permutations. The correlation coefficient between geographic distance and the UniFrac metric estimates was determined using a Spearman's rank correlation conducted in SPSS v.17.

MHC genotyping

DNA was extracted from fin clips using a DNA Wizard Extraction kit (Promega Corp.). The MHC class I-A1 and MHC class II-B1 were amplified using primer sets described in Grimholt *et al.* (1993) and Hordvick *et al.* (1993) respectively. Primers were designed to amplify the peptide binding region located on the α_1 chain of the MHC class I and the β_1 chain of the MHC class II (Grimholt *et al.* 1993; Hordvick *et al.* 1993). We used PCR to amplify each locus following the protocols outlined in Miller *et al.* (1997). The MHC class I-A1 primers amplify either 222 or 228 bp, as a result of a 6 bp deletion in one allelic lineage of the Chinook salmon MHC class I, while the MHC class II-B1 primers amplify 213 bp. For both loci, PCR products were visualized using single strand conformation polymorphism (SSCP). Amplicons were electrophoresed through GeneGels on the Amersham-Biosciences SSCP system using buffer system 'C' at 12 °C following the manufacturer's protocols (GE Healthcare Bio-Sciences Corp.). Gels were then fixed and stained using a silver stain (GE Healthcare Bio-Sciences Corp.). Samples that exhibited unique conformations were cloned using the pGEM T-easy vector kit following the manufacturer's instructions (Promega Corp.). Unique single strand conformations were sequenced at least twice to confirm their allelic make-up. Between four and eight colonies containing inserts were sequenced each time a conformation was sequenced. Alleles that were amplified only once were verified by retyping the individuals containing the allele. Sequencing was conducted at Genome Quebec (McGill University, Quebec, Canada). Sequences were aligned using MEGA and chromatograms were read in FinchTV (Geospiza Inc., http://www.geospiza.com). MHC class I and class II alleles were classified as unique when they encoded for novel amino acids. Estimates of MHC heterozygosity and allele richness within each population and estimates of population genetic divergence (F_{ST}) at the MHC were calculated in FSTAT v. 2.9.3 (Goudet 2002).

MHC and bacterial associations

We examined the relationship between the number of infected and uninfected fish and heterozygosity at the MHC loci using a Fisher's exact tests in SPSS v. 17. We then used multivariate co-inertia analysis (COIA) to examine relationships between the presence/absence of specific MHC class I and class II alleles and the abundance of each bacterial parasite found within individuals (Dolédec & Chessel 1994). The first step in the COIA involved analysing the variation in MHC class I and II genetic data using a principal components analysis (PCA) and then analysing bacterial parasite data in a separate PCA. Examination of the covariance between bacterial and MHC PCAs was then accomplished through the COIA. Associations between MHC alleles and bacteria were assessed by examining the distribution of the factors on the COIA map. The significance of the relationship between the bacterial matrix and MHC matrix was assessed by comparing the covariance between the real data sets with the covariance between 1000 randomly generated data sets. Individuals were pooled across years and across all five populations for these analyses, as it was expected that individual allele–parasite co-evolution should hold across populations. However, to be conservative, we also used a cross-validation approach to re-test the observed allele–parasite associations (Tollenaere *et al.* 2008). For cross-validation, we randomly split the data into two sets and re-ran the COIA on each data set. We considered associations between alleles and bacteria significant if they occurred in both data sets (e.g. Tollenaere *et al.* 2008). Any positive associations between MHC alleles and bacterial phylotypes were taken as evidence of

MHC allele-associated susceptibility to the bacterial parasite. Negative associations between MHC alleles and bacteria were taken as evidence of MHC allele-associated resistance against the given bacterial parasite. All PCA and COIA analyses were conducted in ade4TkGUI within the R statistical environment (Thioulouse & Dray 2007).

Population differentiation (F_{ST}) at the MHC loci was calculated using GENEPOP 3.4 (Raymond & Rousset 1995) and these estimates were combined with previously published estimates based on microsatellite loci (Beacham *et al.* 2006). Mantel tests were then performed in GENEPOP to examine the relationship between the F_{ST} estimates and UniFrac metric estimates of bacterial community similarity. We predicted that, if bacterial communities are sources of selection on the MHC loci, then the MHC F_{ST} estimates, but not the F_{ST} estimates based on the microsatellite loci, would be correlated with the similarity of the bacterial communities across the populations. Unless otherwise indicated, a threshold significance value of 0.05 was used for all statistical tests.

Results

Bacterial identification

We isolated a total of 80 bacterial colonies from the kidney tissues of the Chinook salmon fry. Thirteen of the 80 (16%) colonies did not show a significant similarity to any existing sequence in GenBank; however, the remaining colonies matched existing GenBank sequences with at least 85% similarity and 30 of the 80 (38%) colonies showed more than 97% sequence similarity to existing sequences (Table 1). The number of unique bacterial phylotypes identified within each population ranged from 4 to 21 (Table 1; Supporting information Fig. S1). We identified 21, 19, 7, 7 and 4 bacterial phylotypes from fry from the Big Qualicum, Quinsam, Puntledge, Kitsumkalum, and the lower Skeena populations respectively. A total of 55 unique bacterial phylotypes were identified across populations; only three of the bacterial phylotypes were found in multiple populations (Table 1). In the Quinsam and Kitsumkalum Chinook salmon fry, bacterial phylotypes related to the genus *Pseudomonas* were the most common bacteria identified, exhibiting a prevalence of 3% (3/106) and 5% (3/60) respectively (Table 1). Phylotypes related to the *Mycobacteria* genus and the *Moraxella* genus were the most common bacteria isolated within Big Qualicum River fry, with prevalences of 3% (3/91) each. We isolated a phylotype related to *Yersinia kristensenii* from 10% (2/21) of the fry from the lower Skeena River. In Puntledge River fry, no bacterial phylotype occurred in more than one individual.

Table 2 Bacterial infection rate and bacterial diversity across five populations of Chinook salmon (*Oncorhynchus tshawytscha*). Populations exhibiting significant differences in infection rate or diversity according to a Generalized Linear Model are indicated. Means are reported ± 1 SE

Population	Bacterial infection	Bacterial diversity
Big Qualicum	0.29 ± 0.05*,**	0.33 ± 0.07*,**
Puntledge	0.09 ± 0.03*	0.09 ± 0.03*,***
Quinsam	0.19 ± 0.04	0.19 ± 0.04***
Kitsumkalum	0.15 ± 0.05**	0.15 ± 0.05**
Lower Skeena	0.29 ± 0.10	0.29 ± 0.10

NB: Pairwise examination of the significant differences observed between populations in bacterial infection rate and diversity are indicated by matching symbols. For example, the asterisk in the bacterial infection column indicates a significantly higher bacterial infection rate in the Big Qualicum than the Puntledge River. The significance of the pairwise differences is as follows: Bacterial Infections – *P = 0.001, **P = 0.036; Bacterial Diversity – *P = 0.001, **P = 0.05, ***P = 0.02.

Bacterial community variation

The number of individuals infected by bacteria did not differ between years (GLM $\chi^2_{1,347} = 3.04$, $P = 0.081$; Table 2). However, infection rate did vary significantly among populations (GLM: $\chi^2_{3,368} = 14.6$, $P = 0.002$). Chinook salmon fry from the Big Qualicum and lower Skeena populations exhibited the highest rate of bacterial infection at 29% of individuals (Big Qualicum: 26/90; Skeena: 6/21) and the Big Qualicum population exhibited a significantly higher infection rate than in the Puntledge and Kitsumkalum populations (Table 2). Similarly, bacterial diversity did not vary significantly between years (GLM: $\chi^2_{1,347} = 2.7$, $P = 0.102$), but did vary significantly among populations (GLM: $\chi^2_{3,368} = 16.3$, $P = 0.001$). Again, the Big Qualicum population exhibited higher bacterial diversity than did the Puntledge and Kitsumkalum populations. Moreover, the Quinsam population exhibited higher diversity than did that Puntledge population. The lower Skeena River population was excluded from the analysis of temporal variation in infections as it was not sampled in both years.

Pairwise UniFrac estimates of bacterial community similarity across populations ranged from 0.094 to 0.161, indicating that all populations under comparison exhibit some degree of phylogenetic similarity (Table 3). Big Qualicum and Quinsam rivers were the most similar (0.094) and the Puntledge River and lower Skeena River were the most dissimilar (0.161) in bacterial community. Based on the UniFrac test of community similarity, none of the populations exhibited significantly different phylogenetic community structure

Table 3 UniFrac estimates of bacterial community similarity isolated from five populations of Chinook salmon (*Oncorhynchus tshawytscha*). Pairwise population UniFrac estimates are shown above the diagonal and Bonferonni-corrected significance of the similarity, based on 100 permutations, is shown below the diagonal

	Big Qualicum	Puntledge	Quinsam	Kitsumkalum	Lower Skeena
Big Qualicum		0.098	0.095	0.154	0.160
Puntledge	1.00		0.094	0.145	0.161
Quinsam	1.00	1.00		0.127	0.134
Kitsumkalum	1.00	1.00	1.00		0.127
Lower Skeena	1.00	1.00	1.00	1.00	

(Table 3). Across years, UniFrac estimates of bacterial community similarity were 0.128 in the Kitsumkalum River, 0.155 in the Quinsam River and 0.137 in the Big Qualicum River. Only one bacterial colony was isolated from the Puntledge River in 2006 and it was not successfully sequenced. Therefore, we were unable to examine phylogenetic bacterial community similarity across years in that river. The UniFrac test of community similarity indicated that the bacterial communities did not differ significantly in any of the populations between years. The isolation by distance analysis revealed a significant positive correlation between the UniFrac estimates and geographic distance between populations (Mantel test: $r = 0.88$, $P = 0.012$).

MHC population genetics

Within populations, the MHC class I exhibited between 15 and 27 unique alleles, based on amino acid sequence, and the MHC class II exhibited between 8 and 10 alleles. Across populations, a total of 37 unique alleles were identified at the MHC class I and a total of 17 alleles were identified at the MHC class II (Table 4). Within populations, 50–84% of individuals were heterozygous at the MHC class I, whereas 47–91% of individuals were heterozygous at the MHC class II (Table 4).

MHC and bacterial associations

The proportion of individuals infected with bacteria did not differ between heterozygotes or homozygotes at the MHC class I (Fisher's exact test: $P = 0.65$, $N = 344$; Fig. 2A). However, at the MHC class II the proportion of infected individuals was significantly lower in heterozygotes than in homozygotes (Fisher's exact test: $P = 0.021$, $N = 368$; Fig. 2B). The PCA based on the MHC class I and II allele matrix revealed that the first two axes explained 8.6% of the variance in the data (4.6% F1, 4.0% F2). The first axis (F1) is structured by MHC class I alleles 20, 32, 38, 40 and MHC class II alleles 2, 3, 12 and 13 (Fig. 3A). The second axis (F2) is structured by MHC class II alleles 21 and 22 and MHC class I allele 2. The first and second axes of the bacterial parasite PCA accounted for 9.0% (5.1% F1, 3.9% F2) of the variance in the bacterial matrix data set. The first axis (F1) was structured by *Moraxella osloensis* (AM293373), *Roseomonas* sp. (AY167826), *Arthrobacter* sp. (DQ173023), and *Methylobacterium* sp. (FJ267581; Fig. 3B). The second axis (F2) was structured by *Comamonadaceae* sp. (EF370557) and *Streptomyces* sp. M045 (AY644669).

Major histocompatibility complex and bacterial parasites exhibited significant covariance in the COIA model (global co-inertia=0.24, $P < 0.001$). The first two axes of the COIA model explained 12.6% of the variance shared between the bacteria and MHC matrices (6.9% F1, 5.7% F2). When plotted onto the COIA factor map, five MHC alleles were identified as structuring the co-inertia model. MHC class II alleles 21 and 22 and MHC class I allele 2 were associated with the F2 axis, while MHC class I alleles 28 and 18 were associated with the F1 axis (Fig. 4A). The remaining MHC alleles were located close to the origin of the COIA factor map

Table 4 Summary of genetic variation across five populations of Chinook salmon (*Oncorhynchus tshawytscha*)

Locus		Big Qualicum	Puntledge	Quinsam	Kitsumkalum	Lower Skeena	Total
MHC I	H	0.706	0.704	0.695	0.839	0.500	
	A/A_R	27/16.5	25/15.5	21/13.2	15/10.2	15/15.0	37
MHC II	H	0.778	0.714	0.471	0.915	0.714	
	A/A_R	10/6.3	8/5.6	9/5.1	8/5.6	10/9.9	17

Indicated are the observed heterozygosity (H), number of alleles (A) and allelic richness based on a standardized sample size (A_R), and the total number of unique alleles found across populations at the MHC class I-A1 and class II-B1.

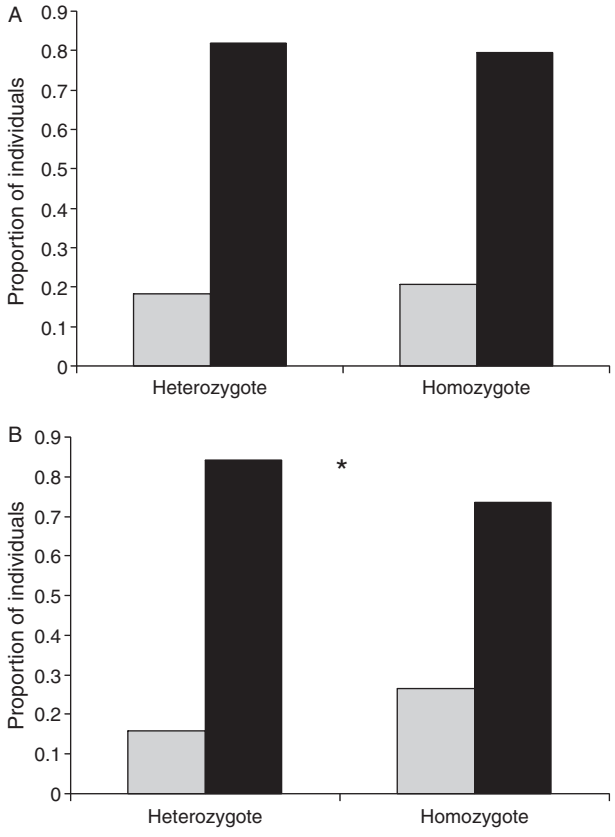

Fig. 2 Occurrence of bacterial infections in MHC homozygous and heterozygous Chinook salmon (*Oncorhynchus tshawytscha*) from five populations in British Columbia, Canada. The proportion of infected (grey) and uninfected (black) fry are indicated for both the MHC class I (A) and MHC class II (B) loci. The asterisk indicates a significant difference in the proportion of heterozygous vs. homozygous fry that were infected with bacteria based on a Fisher's exact test ($P < 0.05$).

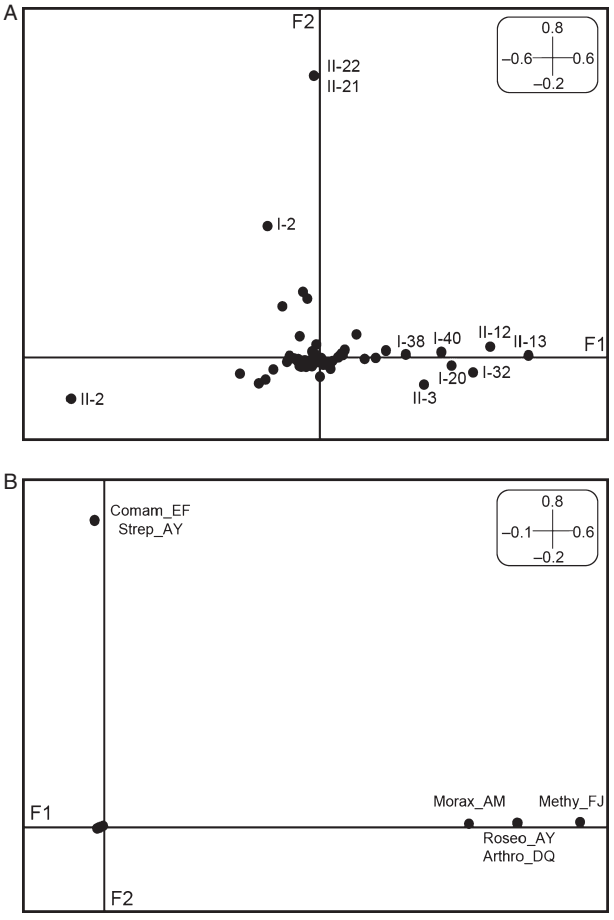

Fig. 3 Principal components analysis (PCA) of (A) MHC genetic variation and (B) bacterial parasites of Chinook salmon (*Oncorhynchus tshawytscha*) fry from five populations in British Columbia, Canada. The MHC class I and class II alleles are indicated as I-allele number and II-allele number respectively. The bacteria variables are indicated by the first five letters of the genus followed by the first two letters of their GenBank match (see Table 1 for full names). Only alleles and bacteria deviating from the mean by more than 0.2 on axis F1 or F2 are labelled.

indicating no strong association between these alleles and bacterial parasites. When considering bacterial associations on the co-inertia factor map, the first axis (F1) was structured by *Moraxella osloensis* (AM293373), *Roseomonas* sp. (AY167826), *Arthrobacter* sp. (DQ173023) and *Methylobacterium* sp. (FJ267581; Fig. 4B). The second axis (F2) was only significantly structured by Alpha proteobacterium (AY642568). The remaining bacteria remained near the origin on the COIA factor map. The co-structure of MHC and bacteria variables on the COIA factor maps indicated that MHC class I allele 2 and MHC class II alleles 21 and 22 were positively associated with infection by the Alpha proteobacterium (AY642568). MHC class I alleles 28 and 18 were positively associated with infection by *Moraxella osloensis* (AM293373), *Roseomonas* sp. (AY167826), *Arthrobacter* sp. (DQ173023) and *Methylobacterium* sp. (FJ267581). We did not detect any antagonistic relationships between MHC alleles and bacteria.

The COIA cross-validation data set 1 confirmed the positive association between Alpha proteobacterium (AY642568) and MHC class II alleles 21 and 22; however, this association was not observed in the COIA of data set 2. The positive association between MHC class I allele 28 and *Moraxella osloensis* (AM293373), *Roseomonas* sp. (AY167826), *Arthrobacter* sp. (DQ173023), and *Methylobacterium* sp. (FJ267581) was confirmed in the COIA of data set 2, but not in data set 1. The associations between MHC class I allele 18 and *Moraxella osloensis* (AM293373), *Roseomonas* sp. (AY167826), *Arthrobacter* sp. (DQ173023) and *Methylobacterium* sp. (FJ267581) and MHC class I allele 2 and Alpha proteobacterium (AY642568) were not confirmed in either data set. The lack of cross-validation of these associations in all data

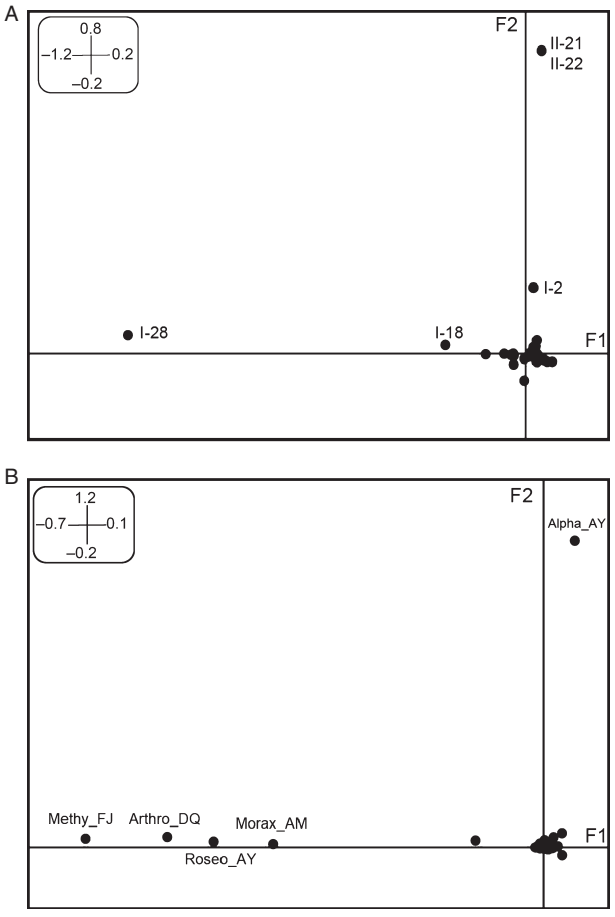

Fig. 4 Co-inertia analysis (COIA) of (A) MHC genetic variation and (B) bacterial parasites of Chinook salmon (*Oncorhynchus tshawytscha*) fry from five populations in British Columbia, Canada. The MHC class I and class II alleles are indicated as I-allele number and II-allele number respectively. The bacteria variables are indicated by the first five letters of the genus followed by the first two letters of the accession number of their GenBank match (see Table 1 for full names). Only alleles and bacteria deviating from the mean by more than 0.2 on axis F1 or F2 are labelled. Variables located in a common direction are positively associated (MHC allele susceptible to bacterial infection). Variables located in the opposite direction are negatively associated (MHC allele resistant to bacterial infection).

sets may be the result of the low prevalence of all bacterial parasites (Table 1) and overall infections in each allele class (Supporting information, Fig. S2). Nevertheless, these associations should be taken as preliminary.

The pairwise population F_{ST} estimates for the MHC loci and the microsatellites are shown in the Supporting information (Table S1). We did not find that pairwise estimates of population genetic divergence (F_{ST}) at the MHC class I or the MHC class II were significantly correlated with UniFrac estimates of bacterial community similarity (Mantel test: MHC class I; $r = 0.28$ $P = 0.299$; MHC class II; $r = 0.25$ $P = 0.098$). Similarly, population genetic divergence at the microsatellite loci was not significantly correlated with UniFrac estimates of bacterial community similarity (Mantel test: $r = 0.49$ $P = 0.098$).

Discussion

Bacterial parasites and community variation

Baseline information on the infection status of wild populations is a necessary first step in understanding the interactions between parasites and hosts (McVicar *et al.* 2006). In this study, we documented an overall bacterial infection rate of 22% and identified a total of 55 unique bacterial phylotypes in Chinook salmon fry from five populations in British Columbia, Canada. Other studies of bacterial parasite diversity in fishes have reported a relatively low number of bacterial parasites in comparison with our study; e.g. 12 bacterial phylotypes in wild Atlantic salmon juveniles (Dionne *et al.* 2009) and eight bacterial phylotypes infecting a variety of wild fish species in Brazil (deSousa & Sila-Sousa 2001). The pathogenicity of most of the bacterial phylotypes identified in our study is not currently understood (see Austin & Austin 2007). However, several of the identified phylotypes are close relatives of known fish pathogens, so it is possible that the infections detected in this study have the potential to affect the health and survival of Chinook salmon fry. For example, congenerics of the *Mycobacteria* phylotype found in both the Big Qualicum and Quinsam populations have been linked to tuberculosis in Chinook salmon in Montana and Oregon (Wood & Ordal 1958; Arakawa & Fryer 1984). Moreover, members of the *Pseudomonas* genus, found in three of the five populations examined in this study, are known pathogens of ayu (*Plecoglossus altivelis*) and rainbow trout (*Oncorhynchus mykiss*; Austin & Stobie 1992; Nishimori *et al.* 2000). These results indicate that infections by a diverse and potentially pathogenic bacterial community are widespread during the early life history stages of Chinook salmon.

Although parasites are recognized as an important component of aquatic ecosystems, relatively little is known about spatial and temporal variation in parasite communities (McVicar *et al.* 2006; Lazzaro & Little 2009). Based on samples collected across consecutive years, we found little evidence of temporal variation in the rate of bacterial infection or the diversity of bacteria infecting Chinook salmon fry. Although some of the UniFrac estimates of phylogenetic community similarity across years were greater than zero, the differences were not statistically significant. Thus, our analysis suggests that the fry are faced with a similar risk of

infection and infections by similar pathogen communities across years. These results are in contrast to those of Dionne *et al.* (2009), who found evidence of temporal variation in bacterial infections in juvenile Atlantic salmon. Although we cannot rule out variation in the pathogen community at a finer temporal scale (i.e. less than 1 year) in Chinook salmon – fry do not meet the minimum size requirements for bacterial analysis during most of their residency in freshwater (see Thoesen 1994) – these results suggest a difference in the temporal stability of bacterial communities between the two species. Few other studies have examined the temporal variation in bacterial parasite communities; thus further studies are needed to fully understand the stability of bacterial communities in fishes.

Examining spatial patterns, the general linear model revealed significant variation in bacterial infection rates and diversity. Dionne *et al.* (2007, 2009) also found evidence of spatial variation in bacterial infection rates across populations of Atlantic salmon. On the other hand, our comparisons based on bacterial community phylogenetic similarity, which take into account the evolutionary relationships between bacterial species, indicated that the Chinook salmon populations exhibit relatively high similarity (UniFrac estimates <0.161). Nevertheless, the isolation by distance pattern of bacterial community similarity suggests that decreasing geographic distance between populations may result in exposure to more similar parasite communities. This pattern may reflect increased dispersal of parasites, more comparable ecological conditions favouring similar bacterial communities, or more recent shared historical distributions of bacterial communities in closely situated populations (Hughes Martiny *et al.* 2006). Our results also suggest that fine-scale spatial variation in bacterial infections occurs within populations as few individuals shared infections by the same bacterial phylotype. Together, our results demonstrate that within and among population variation exists in bacterial infections, and suggest a trend towards diverging parasitic bacterial communities as distance between populations increases.

MHC and bacterial associations

Genetic variation at the MHC is thought to be driven by balancing selection. Our results provide support for heterozygote advantage as a source of balancing selection at the MHC class II in Chinook salmon. The apparent resistance to bacterial infections shown in MHC Class II heterozygotes may result because these molecules play a specific role in the recognition of bacteria (Cresswell 1994). Interestingly, infectivity trials have demonstrated that Chinook salmon MHC class II heterozygous individuals also exhibit higher survival than homozygous individuals, albeit when challenged with infectious hematopoietic necrosis virus (Arkush *et al.* 2002). By contrast, several other studies have linked specific alleles, but not heterozygosity, to infection resistance (e.g. Meyer-Lucht & Sommer 2005; Schad *et al.* 2005; Fraser & Neff 2009), and yet other studies on bank voles (*Myodes glareolus*), water voles (*Arvicola scherman*) and Atlantic salmon found no evidence for heterozygote advantage (Deter *et al.* 2008; Tollenaere *et al.* 2008; Dionne *et al.* 2009). Thus, it remains unclear just how prevalent a heterozygote advantage at the MHC class II locus is across species.

In contrast to the patterns observed at the MHC class II, we did not find evidence of heterozygote advantage at the MHC class I. The MHC class I is typically involved in the recognition of viruses (Cresswell 1994), so it is likely that this locus is not involved in resistance against the bacterial parasites found in our study. Furthermore, other studies of MHC heterozygosity in Chinook salmon have consistently reported a deficit of heterozygotes at the MHC class I locus, which may instead be evidence instead for underdominance (i.e. heterozygous individuals experience lower fitness than homozygotes; Miller *et al.* 1997; Miller & Withler 1997; Garrigan & Hedrick 2001; Heath *et al.* 2006). A recent study in Atlantic salmon suggests that a heteroygote deficiency at the MHC class I in that species is mediated by sperm–egg interactions (Yeates *et al.* 2009). Future studies examining relationships between MHC class I heterozygosity and viral infection may provide insight into the patterns of genetic variation and the potential role of underdominance at this locus.

Negative frequency-dependent selection may also be involved in the maintenance of genetic variation at the MHC if a novel or otherwise rare allele confers fitness benefits to the bearer of that allele (Takahata & Nei 1990; Schad *et al.* 2005). Our co-inertia analysis between MHC alleles and parasites showed a significant global relationship indicating that specific alleles were associated with the prevalence of the various bacteria. However, we found only positive associations between MHC alleles and individual bacterial phylotypes, which indicates MHC susceptibility to specific infections and not the expected resistance. Caution is warranted when interpreting these results because we could not confirm any of the positive associations with a cross-validation analysis. We also did not find that bacterial community similarity was related to population genetic similarity at either of the MHC loci or at microsatellite loci. However, the rarity of each bacterial phylotype and many of the MHC alleles in our populations makes it difficult to detect significant patterns. Thus, our results must be taken as preliminary, but may prove useful in future

studies that aim to examine the role of individual bacterial parasites in MHC evolution.

In conclusion, we detected an unprecedented diversity of parasitic bacteria associated with a vertebrate host, the Chinook salmon, a species that is in decline throughout much of its range (Nehlsen *et al.* 1991). Our results indicate that bacterial parasites are widespread across populations in British Columbia but suggest that levels of bacterial infections are stable across years during the freshwater residency of the host. Within population diversity in the bacterial community was high and is likely to be an important source of balancing selection on MHC genetic diversity. Indeed, we found evidence of a heterozygote advantage at the MHC class II locus. Moreover, variation in bacterial infection rates across populations and significant isolation by distance in the phylogenetic similarity of bacterial communities imply spatial variation in selection pressures by these parasites. Our results thereby make a novel contribution to the understanding of host–parasite relationships within and among populations of a vertebrate species.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Pairwise population genetic divergence (F_{ST}) estimates at the MHC class I, MHC class II and 13 microsatellite loci in five Chinook salmon populations (BQ, Big Qualicum; QR, Quinsam River; PR, Puntledge River, LS, Lower Skeena; KR, Kitsumkalum River) in British Columbia, Canada

Fig. S1 Phylogenetic trees of bacterial 16s rDNA sequences obtained from kidney tissue of Chinook salmon (*Oncorhynchus tshawytscha*) from five populations in British Columbia, Canada. Trees show the sequence relationships between the bacteria isolated from each population and the sequence of the closest relative in GenBank. Neighbour-joining trees and bootstrap support values are based on 500 repetitions performed in MEGA v.4. The populations comprise: Quinsam River (A), Big Qualicum River (B), Puntledge River (C), Kitsumkla.

Fig. S2 MHC allele frequencies and bacterial infections in Chinook salmon (*Oncorhynchus tshawytscha*) from five populations in British Columbia, Canada. Bars separately denote the relative allele frequencies in uninfected (black) and infected (grey) fry for the MHC class I (A) and class II (B) loci [i.e. the overall frequency of each allele (of a total of 1) is shown separately for infected and uninfected fish].

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NEWS AND VIEWS

PERSPECTIVE

Pathogens as potential selective agents in the wild

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Pathogens are considered a serious threat to which wild populations must adapt, most particularly under conditions of rapid environmental change. One way host adaptation has been studied is through genetic population structure at the major histocompatibility complex (MHC), a complex of adaptive genes involved in pathogen resistance in vertebrates. However, while associations between specific pathogens and MHC alleles or diversity have been documented from laboratory studies, the interaction between hosts and pathogens in the wild is more complex. As such, identifying selective agents and understanding underlying co-evolutionary mechanisms remains a major challenge. In this issue of *Molecular Ecology*, Evans & Neff (2009) characterized spatial and temporal variation in the bacterial parasite community infecting Chinook salmon (*Oncorhynchus tshawytscha*) fry from five populations in British Columbia, Canada. They used a 16S rDNA sequencing-based approach to examine the prevalence of bacterial infection in kidney and looked for associations with MHC class I and II genetic variability. The authors found a high diversity of bacteria infecting fry, albeit at low prevalence. It was reasoned that spatial variability in infection rate and bacterial community phylogenetic similarity found across populations may represent differential pathogen-mediated selection pressures. The study revealed some evidence of heterozygote advantage at MHC class II, but not class I, and preliminary associations between specific MHC alleles and bacterial infections were uncovered. This research adds an interesting perspective to the debate on host–pathogen co-evolutionary mechanisms and emphasizes the importance of considering the complexity of pathogen communities in studies of host local adaptation.

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In the context of frequent environmental changes, pathogens may represent selective agents promoting local adaptation in host populations through adaptive genetic variation. Growing evidence now exists for pathogen-mediated selection on genes of the major histocompatibility complex (MHC) (reviewed in Sommer 2005; Piertney & Oliver 2006). However, the underlying mechanisms involved in pathogen resistance are not well understood and the identification of selective agents in the wild remains in its infancy. In this study, Evans & Neff (2009) examined the diversity and structure of bacterial communities infecting Chinook salmon fry populations in the wild (Fig. 1). They found a high diversity of bacteria infecting fry, with 55 unique phylotypes identified over the five populations studied. This estimate, which is probably conservative because only certain types of bacteria can grow under the studied conditions (agar plates under aerobic conditions), suggests the occurrence of a higher than expected diversity of bacteria infecting Chinook salmon at the beginning of its life cycle when freshwater mortality can be high (Healey 1991). The prevalence of bacteria was relatively low, which is similar to results from other juvenile salmonids (Dionne *et al.* 2009) and could represent epizootic pathogens, affecting a limited number of fish but having the capacity to spread rapidly in populations under favourable environmental conditions. The freshwater pathogenic or potentially pathogenic bacteria identified in Chinook salmon from this study constitute a necessary first step to understand host-pathogen interactions in the wild.

Evans & Neff (2009) identified spatial variability in both the number of bacterial phylotypes and infection rates across populations (phylotypes: 4–21; infection rates: 10–29%), with no apparent geographical trend. Interestingly, infection rates in salmon inhabiting the main stem and one tributary of the Skeena River were mildly different, but not significantly, potentially suggesting some variability in host-pathogen interactions within a river system. The spatial variability in infection rates and bacterial diversity detected in kidneys of Chinook salmon in this study could be mediated by environmental selection pressures differing among habitats or by variance among populations in immune resistance, potentially related to genetic variability at the MHC. Indeed, high infection rates in host individuals could be the result of a high selection pressure from the environment or a low immune resistance capability, or both as previously observed in Atlantic salmon (*Salmo salar*; Dionne *et al.* 2009). In future studies, it will be interesting to differentiate between these two hypotheses by quantifying selection pressure from the host environment through the identification of pathogens in water and soil, for example, in the case of fish populations.

A UniFrac metric of phylogenetic community similarity was used to compare bacterial communities infecting fish across populations and years (Evans & Neff 2009). This

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Fig. 1 Infected male Chinook salmon (*Oncorhynchus tshawytscha*). Elwha River, Washington State. (Photo: John McMillan.)

adds a new and interesting perspective on how pathogen community composition, in addition to pathogen diversity and richness, might vary according to geographical distance. They found relatively low weighted UniFrac estimates (spatial scale: 0.094–0.161; temporal scale: 0.128 to 0.155) suggesting a certain level of similarity in pathogen communities that infect Chinook salmon through space and time. Interestingly, an isolation by distance pattern in bacterial community phylogenetic similarity was uncovered across infected populations, which could suggest differential selection pressure as geographical distance increases. However, as southern populations were sampled earlier in the summer than northern populations, short-term variation in bacterial community cannot be excluded to explain this pattern. On the other hand, no significant change in bacterial community composition was observed between the two consecutive years of the study in three populations, suggesting a certain stability over a longer temporal scale. Clearly, this shows how diverse and complex bacterial communities might be in the wild and emphasizes the need to consider pathogen community composition through space and time when studying host adaptation.

When relating infection rates and MHC genetic variability, Evans & Neff (2009) found some evidence of heterozygote advantage at MHC class IIβ, but not at MHC class Iα. Indeed, the proportion of infected individuals was significantly lower for MHC class II heterozygotes than for MHC class II homozygotes. These findings support the role of MHC class II in bacterial resistance and are concordant with balancing selection imposed by pathogens. The authors also found associations between MHC class I and II alleles and the prevalence of specific bacteria in kidney through a co-inertia (COIA) analysis showing significant covariance between MHC alleles and individual bacterial abundance matrices (12.6% variance explained by the first two axes of the COIA model). However, these associations were not all cross-validated by a second analysis, possibly because of the low bacterial prevalence and infection rate observed within the studied populations, as suggested by the authors. Nevertheless, these represent preliminary analyses identifying potential susceptibility alleles towards some infections and could help orient future work on pathogen resistance in a context of multiple pathogen exposure. Such associations between specific MHC alleles and the prevalence of infections was previously observed in multiple vertebrates including humans (e.g. Hill *et al.* 1991), chickens (e.g. Briles *et al.* 1977) and salmonids (e.g. Langefors *et al.* 2001), and support the frequency-dependent selection and the variable selection in time and space hypotheses (Nei & Hughes 1991; Hedrick 2002). In the literature so far, frequency-dependent selection has received more support as a mechanism of balancing selection maintaining MHC diversity in wild populations (Sommer 2005). This study adds another perspective to the debate on host-pathogen interactions and underlines the importance of conducting such studies in the wild to complement existing controlled laboratory experiments.

Understanding mechanisms underlying host-pathogen interactions remains a major challenge and will certainly continue to offer exciting opportunities for future research. The study of Evans & Neff (2009) brings an interesting perspective on how diverse and complex pathogen communities infecting fish might be in the wild and underlines the importance of considering this complexity in host local adaptation studies. This research also identified potential selective agents and related their prevalence to host adaptive genetic variability, and as such, took us one step closer to reaching a global understanding of pathogen resistance and host adaptation in the wild.

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