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CLINAL VARIATION IN MHC DIVERSITY WITH TEMPERATURE: EVIDENCE FOR THE ROLE OF HOST–PATHOGEN INTERACTION ON LOCAL ADAPTATION IN ATLANTIC SALMON

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In vertebrates, variability at genes of the Major Histocompatibility Complex (MHC) represents an important adaptation for pathogen resistance, whereby high allelic diversity confers resistance to a greater number of pathogens. Pathogens can maintain diversifying selection pressure on their host's immune system that can vary in intensity based on pathogen richness, pathogen virulence, and length of the cohabitation period, which tend to increase with temperature. In this study, we tested the hypothesis that genetic diversity of MHC increases with temperature along a latitudinal gradient in response to pathogen selective pressure in the wild. A total of 1549 Atlantic salmon from 34 rivers were sampled between 46°N and 58°N in Eastern Canada. The results supported our working hypothesis. In contrast to the overall pattern observed at microsatellites, MHC class II allelic diversity increased with temperature, thus creating a latitudinal gradient. The observed temperature gradient was more pronounced for MHC amino acids of the peptide-binding region (PBR), a region that specifically binds to pathogens, than for the non-PBR. For the subset of rivers analyzed for bacterial diversity, MHC amino acid diversity of the PBR also increased significantly with bacterial diversity in each river. A comparison of the relative influence of temperature and bacterial diversity revealed that the latter could have a predominant role on MHC PBR variability. However, temperature was also identified as an important selective agent maintaining MHC diversity in the wild. Based on the bacteria results and given the putative role of temperature in shaping large-scale patterns of pathogen diversity and virulence, bacterial diversity is a plausible selection mechanism explaining the observed association between temperature and MHC variability. Therefore, we propose that genetic diversity at MHC class II represents local adaptation to cope with pathogen diversity in rivers associated with different thermal regimes. This study illuminates the link between selection pressure from the environment, host immune adaptation, and the large-scale genetic population structure for a nonmodel vertebrate in the wild.

KEY WORDS: **Coevolution, genetic diversity, local adaptation, MHC, salmon, temperature.**

Since the middle of the 20th century, infectious diseases have been identified as an important selection force in the evolution of animals (Haldane 1949). Pathogens are increasingly emerging in natural populations and infectious diseases are considered a serious threat to small or endangered populations by their potentially disastrous effect on demography (Lafferty et al. 2004; Acevedo-Whitehouse and Cunningham 2006). Pathogen infectivity and virulence are influenced by many factors that can vary spatially with

environmental conditions, in particular temperature. There is evidence that pathogen richness and diversity increase from the poles to the equator in concordance with the "latitudinal diversity gradient" (LDG) observed in many taxonomic groups (Hawkins and Porter 2001; Kuklinski et al. 2006; Stevens 2006). This trend has been documented for infectious diseases in humans (Guernier et al. 2004) and for ectoparasites in fish (Rohde and Heap 1998). Temperature is potentially a major factor in generating this variation in species diversity (Clarke and Gaston 2006) through its effect on metabolism (Gillooly et al. 2001; Allen et al. 2002) and indirect impact on generation time and mutation rates (Gillooly et al. 2005; Allen et al. 2006). Temperature also plays a role in determining the level of infectivity and virulence of pathogens (Griffiths 1991). For many pathogens, infectivity and virulence increase with temperature, as demonstrated for pathogenic bacteria associated with different fish species (Nordmo and Ramstad 1999; Larsen et al. 2004; Zheng et al. 2004). Finally, infectivity and virulence of pathogens can also be influenced by the length of the host–pathogen cohabitation period. In environments with long summers, pathogen exposure can occur over an extended period of time, which can lead to enhanced selection for pathogen infectivity and virulence (André and Day 2005). Overall, these studies suggest that pathogens should be more diverse, more virulent, and in contact with their host for a longer period of time in warmer than in colder environments. This in turn raises the hypothesis that temperature creates a gradient in pathogen selection pressure increasing from northern cold to southern warm latitudes in the northern hemisphere.

In vertebrates, genes of the Major Histocompatibility Complex (MHC) are involved in the immune response by encoding cell-surface proteins that bind peptide fragments derived from pathogens and display those fragments to T-cells that activate an appropriate immune response (Potts and Wakeland 1990). These genes are among the most polymorphic in vertebrates, with most of the variability concentrated at the peptide-binding region (PBR) involved in pathogen's binding (Hughes and Yeager 1998). Variation of only one or a few amino acids in the PBR can lead to large differences in the repertoire of peptides bound, and hence to pathogen resistance (Frank 2002; Schad et al. 2005). At the individual level, some alleles have been shown to confer resistance to specific diseases such as malaria in humans (e.g., Hill et al. 1991), Marek's disease in chickens (Briles et al. 1977), and furunculosis in the Atlantic salmon (Langefors et al. 2001; Grimholt et al. 2003). At the population level, high MHC diversity is advantageous for conferring resistance to a diverse array of pathogens on an evolutionary time frame and this diversity is thought to be maintained by balancing selection, through heterozygote advantage, frequency-dependent selection, or variable selection in time and/or space (Nei and Hughes 1991; Hedrick 2002). In the wild, selection can change spatially as a function of local parasite communities (Thompson 1994; Hedrick 2002), however, clear evidence of an association between large-scale variation in selection regime and MHC diversity is limited to humans (Prugnolle et al. 2005). Indeed, most studies conducted to date cannot reject the hypothesis that spatial patterns of MHC diversity are mainly governed by demographic processes and population structure (Piertney and Oliver 2006). To better understand the evolutionary importance of MHC genes for vertebrate local adaptation, there is a need for studies conducted in the wild which could (1) differentiate between natural selection and other evolutionary forces, (2) identify potential selective agents influencing MHC diversity, and (3) provide evidence for or against the putative role of pathogens as a selective pressure at a large spatial scale (Bernatchez and Landry 2003; Sommer 2005; Piertney and Oliver 2006).

Given the ample empirical evidence of the association between temperature and pathogen diversity and virulence, the objective of this study was to assess the role of temperature on the maintenance of MHC gene diversity along a latitudinal gradient in wild populations of Atlantic salmon, *Salmo salar*. Anadromous populations of Atlantic salmon reproduce and spend the first years of their life in rivers along the North American and the European Atlantic coasts. Populations have been declining for several decades and some (e.g., Inner Bay of Fundy populations) have been identified as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Within the North American range of Atlantic salmon distribution, we hypothesized that the strength of balancing selection from pathogens in the wild should increase from cold to warm environments, resulting in an increase in MHC diversity from cold northern to warm southern habitats. To disentangle the relative role of selection versus demographic processes in maintaining this pattern, we compared MHC class II gene diversity with neutral expectations at two genetic levels: the allelic frequency and the amino acid composition levels. Comparing a functional gene potentially under selection, such as MHC genes, with neutral molecular markers influenced by neutral evolutionary forces alone (gene flow, drift, and mutation) allows isolating the potential influence of selection acting on that candidate gene (Bernatchez and Landry 2003; van Oosterhout et al. 2006). We then tested the predictions that (1) at the allele level, MHC class IIβ diversity will increase more strongly with temperature than diversity at neutral microsatellite markers; (2) at the amino acid level, MHC class IIβ diversity within the PBR involved in pathogen binding will increase more strongly with temperature than diversity at putative neutral sites (non-PBR). To infer a more direct association between patterns of MHC diversity and selection imposed by bacterial diversity, for a subset of populations, we tested the prediction that (3) diversity of the PBR will increase more strongly with bacterial diversity than diversity of the non-PBR.

Methods

SAMPLING

A total of 1549 adult salmon were sampled during summer 2004 through sport fishing in 34 rivers in Québec and Labrador, Canada, located from 46°N to 58°N latitude and subjected to different temperature regimes (see online Supplementary Appendix S1). Salmon were measured and their adipose fins clipped and stored in 95% ethanol for the DNA analysis.

MICROSATELLITES AND MHC CLASS IIβ GENOTYPING

DNA was extracted from fin clips using the Qiagen DNeasy Tissue Kit (Mississauga, Ont, Canada) following the manufacturer's guidelines Microsatellite polymorphism was quantified at 13 loci using fluorescently labeled primers: *Ssa85*, *Ssa202*, *Ssa197* (O'Reilly et al. 1996), *Ssosl417* (Slettan et al. 1995), *SsaD71*, *SsaD85*, *SsaD144* (T. King, unpubl. ms.), *MST-3* (Presa and Guyomard 1996), *Sssp1605*, *Sssp2210*, *Sssp2215, Sssp2216*, and *SsspG7* (Paterson et al. 2004). MHC class IIβ exon 2 was amplified using the forward primer 5′-ACC TGT CTT GTC CAG TAT GG-3′and the reverse primer 5′-TGC CGA TAC TCC TCA AAG GAC-3′ (Rox labeled). A GC-clamp was attached to the 5′ end of the forward primer to aid in separation of the alleles via denaturing gradient gel electrophoresis (DGGE) (5′-GCC CGC CCC CGC CGC CCA GCC CGC GCC CCG CGC CGC CCG CGG). Microsatellites and MHC amplification and genotyping conditions are detailed in Appendix S2 (see online Supplementary Material).

BACTERIAL DIVERSITY ANALYSES IN RIVERS

To obtain preliminary evidence of a direct association between host MHC diversity and pathogen selection pressure along the temperature gradient, we analyzed the bacterial communities from the water of seven of the 34 rivers located between 48°N and 50°N latitude. Bacterial diversity was evaluated for each river to represent an index of the selection pressure present in the environment of the fish. This index is meant to reflect the level of challenges experienced by the immune system of the fish over an evolutionary time scale by including both pathogenic and potentially pathogenic bacteria. This contrasts with other studies that have examined fish infection levels that represent both environmental selection pressure and individual resistance capabilities, both factors being confounded (Wegner et al. 2003; Šimková et al. 2006). One to three water samples were collected per river at three time periods between June and August. For all samples, water was filtered, bacterial DNA was extracted and amplified using a nested PCR protocol, and bacterial sequences were separated using the DGGE method (see online Supplementary Appendix S2 for the detailed method). The time required, in the field and in the laboratory, to set up and run this new technique only allowed us to include a subset of the rivers in the analysis. Global bacterial diversity was assessed for each water sample, which represents pathogenic, potentially pathogenic, and nonpathogenic bacteria. Most bacteria causing disease in fish are opportunistic pathogens that are present as part of the normal water microflora, at least in marine environments (Hansen and Olafsen 1999) and species biodiversity in an ecosystem is closely related to pathogen diversity (Hudson et al. 2006). Hence, overall bacterial diversity was used as a surrogate for pathogenic and potentially pathogenic bacterial diversity to represent the relative evolutionary selection pressure present in the different river environments.

STATISTICAL ANALYSES

For both microsatellite and MHC loci, allele richness (A), adjusted for the sample size of the smallest population (34 individuals), was calculated for each population using FSTAT version 2.9.3.2 (Goudet 2001). Deviation from Hardy–Weinberg expectations, linkage disequilibrium, observed (Ho), and expected (He) heterozygosity per locus and per population were calculated using Genepop 3.4 (Raymond and Rousset 1995). The potential occurrence of null alleles and scoring errors due to stuttering or large allele dropout in the dataset was assessed using the software Micro-Checker (van Oosterhout et al. 2004).

Genetic diversity at the allelic level

To test for the presence of a temperature gradient in genetic diversity at MHC class IIβ, a regression model was performed between allelic richness and temperature. Furthermore, to examine if the influence of temperature creates a latitudinal gradient in genetic diversity in this system, a regression model was also performed between allelic richness and latitude (river mouth for each population). Temperature was estimated using a historical temperature index based on the number of degree-days above 5°C (temperature that is encountered from spring to fall in the study area and that covers the growth season of salmon) for each month between April and October and cumulated over a 30-year period (1971–2000, Environment Canada, http://climate.weatheroffice.ec.gc.ca/climateData/canada_f.html). To test the neutral hypothesis that the temperature pattern of MHC diversity was not affected by selection, we compared the relationship between MHC allelic diversity and temperature with that observed at microsatellite markers. We first performed the LnRH neutrality test to assess whether some of the microsatellites used might themselves be subjected to selection, for example, by genetic hitchhiking (Kauer et al. 2003). For each locus, the LnRH test calculates the ratio of gene diversity between two populations and considers the joint empirical distribution of all loci to identify loci that differ significantly in variability from the remainder of the genome (Schlötterer 2002). This test is independent of mutation rates and is relatively insensitive to demographic events (Kauer et al. 2003). Maple version 7.0 (2001) was used to calculate the gene diversity

ratio between all possible pairs of populations and a Fisher F-statistic was used to identify the loci that differed significantly from neutrality in their gene diversity ratios. Gene diversity (Nei 1987) was calculated using FSTAT version 2.9.3.2. To further account for the potential effect of demography, colonization history, and restricted gene flow on MHC diversity, two additional tests were conducted. First, the potential influence of demography was evaluated by estimating the strength of the relationship between MHC diversity and the effective population size (N_e) using a linear regression model. Effective population size was calculated based on the neutral microsatellite markers using the linkage disequilibrium method implemented in NeEstimator (Peel et al. 2004). Second, to evaluate the potential influence of colonization history and restricted gene flow on MHC diversity, isolation by distance (IBD) was tested by comparing genetic distance ($F_{ST}/1 - F_{ST}$) and geographic (latitudinal) distance for both MHC and neutral markers using the IBD 1.52 software (Bohonak 2002). F-statistics were calculated using Genepop 3.4 (Raymond and Rousset 1995). Latitude was used to represent geographic distance because Atlantic salmon colonization is hypothesized to have occurred from south to north in the study area after the retreat of the Wisconsin Ice Sheet (Crossman and McAllister 1986).

Genetic diversity at the amino acid level

MHC sequences were aligned using BioEdit 7.0.5.3 (Hall 1999). To compare the effect of selection on PBR codons involved in pathogen binding versus on putatively pathogen-neutral non-PBR codons, Z-tests of selection were performed using Mega 3.1 (Kumar et al. 2000) by comparing nonsynonymous (*dn*) and synonymous (*ds*) substitutions (Nei and Gojobori 1986). These tests were performed separately for codons of the putative PBR and for the non-PBR codons, as well as for all sites combined. The PBR was identified based on codons involved in pathogen binding in humans (Brown et al. 1988, 1993) and corresponds to those presented in Landry and Bernatchez (2001). To test the temperature selection hypothesis at the amino acid level, MHC class IIβ amino acid diversity for each population was estimated for PBR and non-PBR codons separately using the diversity index d calculated using the DIVAA software (Rodi et al. 2004). This index calculates the proportion of each amino acid present at each codon from aligned sequences following the equation:

$$d = 1/\left(N \sum p_k^2\right),$$

where N is the number of possible amino acids at a given position (i.e., 20) and p_k is the probability of finding the kth amino acid at that position. Thus, a highly conserved position will have a low index value and reciprocally, a highly polymorphic position will have a high index value. Regression models between the MHC amino acid diversity index d for each population and temperature were performed for the putative PBR and non-PBR separately to test the null hypothesis of no difference in MHC diversity gradient associated with temperature between these two molecular regions. A discrepancy between PBR and non-PBR diversity gradient with temperature would provide evidence concerning the intensity of selection acting specifically on pathogen binding sites of the MHC class IIβ gene. To evaluate the potential selective impact of pathogens from the environment, the same analyses were performed between d and the bacterial diversity index to test the null hypothesis of no difference in MHC diversity gradient associated with bacteria diversity between the PBR and the non-PBR. For all regression models, the type of regression that best explained the variability of the data was assessed using a model selection approach based on Akaike information criterion (AIC). Homogeneity of variances was tested using Cochran's C-test and normality of data was assessed by examining plots of the residuals. Finally, to compare the relative influence of temperature versus bacteria diversity in explaining MHC amino acid diversity at PBR, a model selection approach based on AIC was performed on the seven rivers for which bacteria diversity data were available. AIC corrected for small sample sizes (AICc) was calculated for each model (logarithmic regression, PROC MIXED) according to Burnham and Anderson (2002). The most parsimonious fit to the data was considered as the model with the smallest ΔAIC_C and the largest AIC_C weight. Regressions and model selection analyses were performed using SAS software (ver. 9.1 SAS Institute).

Results

GENETIC POLYMORPHISM

The total number of alleles per microsatellite locus was: *Ssa85*: 25; *Ssa197*: 21; *Ssa202*: 22; *SsaD71*: 39; *SsaD85*: 37; *SsaD144*: 53; *Ssosl417*: 29; *Sssp1605*: 15; *Sssp2210*: 15; *Sssp2215*: 19; *Sssp2216*: 38; *SsspG7*: 26; *MST-3*:13 (mean of 20.7 alleles per locus). The mean number of alleles per population averaged over the 13 microsatellites ranged from 11.5 to 16.9 alleles (see online Supplementary Appendix S1). Sixteen alleles were found at MHC class IIβ, with a mean of 10.7 alleles per population. MHC alleles differed on average by 8.7 amino acid substitutions (range: 2–17). Mean observed heterozygosity was 0.84 for microsatellites (range: 0.74–0.89) and 0.85 for MHC (range: 0.72–1.00) (see online Supplementary Appendix S1). For microsatellites, 27 of 442 comparisons showed evidence of significant deviations from Hardy–Weinberg proportions (all heterozygote deficits), a number similar to the 22 significant tests expected by chance at $\alpha = 0.05$. There was no evidence of significant deviations associated either with particular loci or populations. For MHC, a significant deviation from Hardy-Weinberg proportions was found in three populations (Koksoak, Matane, and Trinité), again a number close to the two significant tests expected by chance at $\alpha = 0.05$.

When comparing linkage disequilibrium between MHC and microsatellite loci, one comparison, MHC and *Ssa202*, was significant (one significant test is expected at $\alpha = 0.05$). There was no significant pattern of linkage disequilibrium within any of the populations or between any locus pairs across populations for microsatellites. Potential null alleles were suggested at only three population-loci combinations out of 442, with the estimated null allele frequency as follows: Gros Mecatina River at locus *Sssp2210* (0.10), Southwest River at locus *Ssosl417* (0.13) and York River at locus *Ssa85* (0.10). No evidence for scoring errors due to stuttering or large allele dropout was found in the whole dataset. We therefore concluded that neither potential null alleles nor scoring errors affected the outcome of the results.

MHC AND MICROSATELLITE ALLELIC DIVERSITY

The LnRH neutrality test revealed that microsatellite *Sssp1605* was a significant outlier in terms of gene diversity relative to other loci ($P < 0.0001$). Given that this microsatellite marker is potentially under selection, it was excluded from the remainder of the analyses. Temperature above 5°C cumulated from April to October and averaged over the period 1971–2000 ranged from 1797.8 degree-days for the Jacques-Cartier River to 531.8 degree-days for rivers in the Ungava Bay region (see online Supplementary Appendix S1). MHC allelic richness increased significantly with temperature ($r^2 = 0.37, P = 0.0001$; logarithmic regression model: ωAICc = 0.999; Fig. 1A). Temperature and latitude were highly correlated in this system ($r^2 = -0.78, P < 0.0001$), which resulted in a decrease in MHC allelic richness with latitude ($r^2 = 0.28, P = 0.001$; logarithmic regression model: ωAICc = 0.992; Fig. 1B). In contrast, allelic richness for all microsatellites combined did not vary significantly with temperature ($r^2 = 0.009$, $P = 0.06$, Fig. 2). When analyzed individually, allelic richness for 10 of 12 microsatellites did not vary significantly with temperature ($r^2 = 0.07 \pm 0.07$, $\alpha = 0.05$ following sequential Bonferroni correction), whereas *SsaD71* and *Ssosl417* increased significantly with temperature ($r^2 = 0.27$ and 0.39, respectively, $P < 0.004$). Effective population size (N_e) ranged from 52 in the Southwest River, Labrador, up to 1112 in the Matapedia River, Gaspésie (see online Supplementary Appendix S1). The effective population size of the Ouelle and the Moisie populations could not be estimated by the software, but indicated a very low (near zero) and a very high (infinite, min. 95% CI = 1953) effective population size, respectively. Therefore, they were excluded from the effective population size analysis. MHC allelic richness did not show any significant relationship with effective population size ($r^2 = 0.02$, $P = 0.5$). When comparing genetic distance and latitude matrices, no significant IBD pattern was observed for MHC ($r^2 = 0.02, P = 0.2$), in contrast with the highly significant IBD pattern observed for all microsatellites combined ($r^2 = 0.43, P = 0.001$). When looking at microsatellite loci individually, 10 loci out of 12 showed a significant pattern of IBD, including *SsaD71* and *Ssosl417* ($\alpha =$ 0.05 following sequential Bonferroni correction).

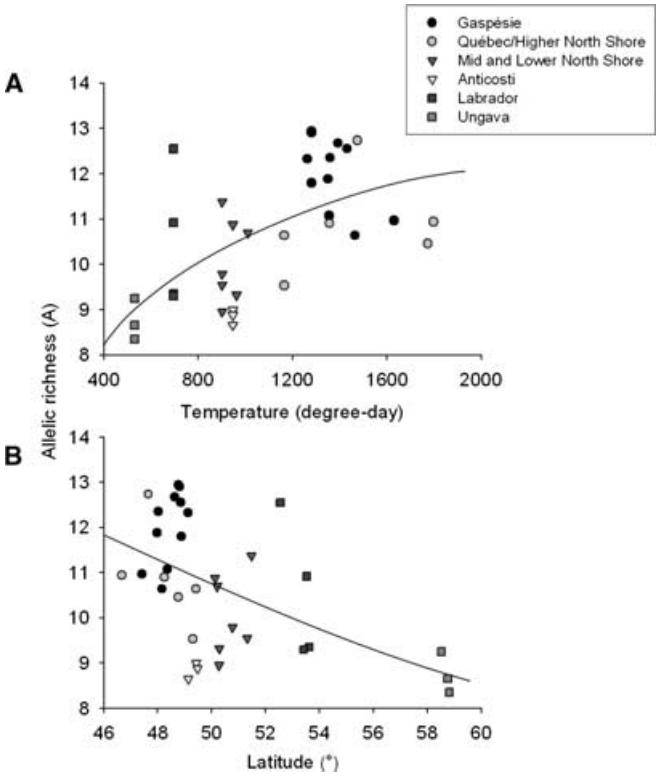

Figure 1. Allelic richness (A) at MHC class IIβ for Atlantic salmon populations experiencing different temperatures (A) and located at different latitudes (B). Each geographical region is indicated by a different symbol.

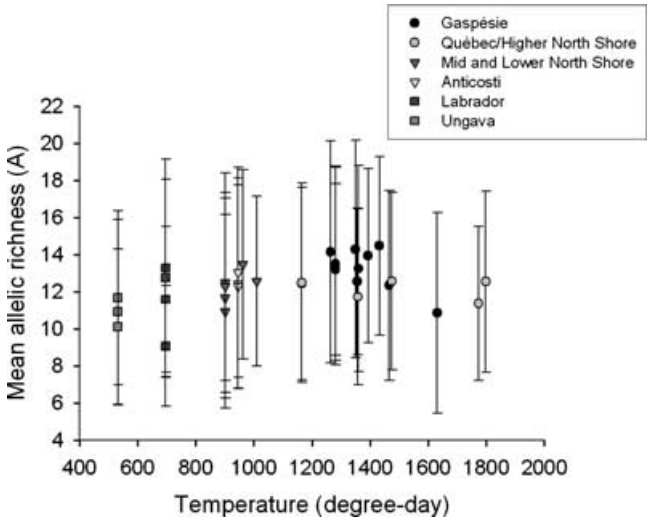

Figure 2. Mean allelic richness per population (A) at 12 microsatellite loci for Atlantic salmon populations experiencing different temperatures. Vertical bars represent standard deviation. Each geographical region is indicated by a different symbol.

MHC AMINO ACID DIVERSITY

When comparing MHC sequences, the ratio of nonsynonymous (*dn*) to synonymous (*ds*) substitutions at the PBR was significantly different than neutral expectation (1:1 ratio), and was 4.9 times higher than the ratio at the non-PBR. This ratio was also significant for all sites combined (Table 1). MHC amino acid diversity per site ranged from 0.05 (conserved site) to 0.18 (the most polymorphic site) and was significantly higher for PBR than for non-PBR codons (*t*-test: $P < 0.0001$, Fig. 3A). MHC amino acid diversity at the PBR for each population showed a significant logarithmic increase with temperature ($r^2 = 0.24$, $P < 0.001$; logarithmic regression model: ωAICc > 0.999), whereas no significant relationship between amino acid diversity and temperature was observed for the non-PBR ($r^2 = 0.03$, $P = 0.3$; Fig. 3B). The analysis of amino acid diversity further revealed that three specific amino acids out of 18 in the PBR had the highest regression coefficient and were driving the positive relationship between MHC class II diversity and temperature. These corresponded to positions 58 ($r^2 = 0.27$, $P = 0.002$), 72 ($r^2 = 0.18$, $P = 0.01$), and 73 ($r^2 = 0.34$, $P < 0.001$, Fig. 3A) of the MHC class IIβ exon 2 sequence. Position 73 was the most polymorphic amino acid position.

BACTERIAL DIVERSITY IN RIVERS

The number of DGGE bands in each water sample averaged 7.2 and ranged from two to 17, for a total of 28 different bands detected. This is comparable to other bacterial studies using DGGE for diversity estimation (for a review, see Troussellier et al. 2002). The bacterial diversity index per river, based on the number and the fluorescence intensity of the bands, was 21.7×10^3 IOD/mm^2 on average and ranged from 0.3×10^3 to 53.6×10^3 IOD/mm^2 (see online Supplementary Appendix S2 for more details on index calculation). The seven rivers for which bacterial diversity was assessed were located in a restricted temperature range, varying from 1009.6 to 1361.5 degree-days (28% of the initial temperature range). Within this temperature range, a significant logarithmic increase in MHC amino acid diversity with bacterial diversity was observed at the PBR ($r^2 = 0.70$, $P = 0.02$; logarithmic regression model: ωAICc = 0.992), but not at the non-PBR ($r^2 = 0.57$, $P = 0.05$, Fig. 3C), although the regression coefficient of the later was relatively high. The analysis based on the seven rivers revealed that "bacteria diversity" was selected as the best model explaining MHC PBR variability ($\Delta AIC_C = 0$; ωAICc = 0.559). However, "temperature" was also a good model for predicting MHC PBR variability ($\Delta AIC_C = 1.1$; ωAICc = 0.323, Table 2).

Table 1. Ratio of nonsynonymous (*dn*) to synonymous (*ds*) substitutions for MHC class IIβ for the peptide-binding region (PBR), other regions (non-PBR), and all sites combined. The number of codons for each category and the *P* values testing departure from neutrality are indicated.

Region	Codon	*dn*/*ds*	*P*
Non-PBR	64	0.988	0.329
PBR	18	4.917	<0.001
Total	82	3.065	0.003

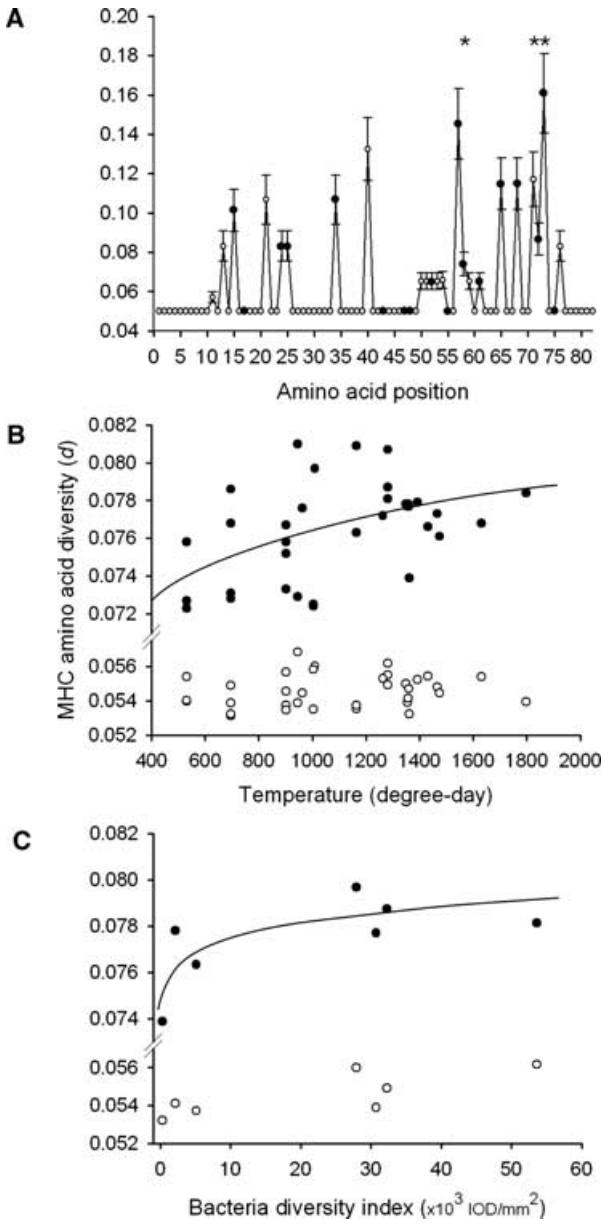

Figure 3. Amino acid diversity (*d*) at MHC class IIβ for the peptide-binding region (PBR, black circles) and non-peptide-binding region (non-PBR, opened circles) for Atlantic salmon populations. Amino acid diversity (*d*) is represented for (A) each site when all MHC sequences from all populations are compared, (B) each population situated at different temperatures and (C) seven populations living with different bacteria diversity communities. Asterisks in (A) represent positions 58, 72, and 73 that have the strongest diversity relationship with temperature. Note the different amino acid diversity levels between PBR and non-PBR indicating a higher polymorphism for PBR than for other sites.

Table 2. Model selection analysis comparing the influence of bacteria diversity and temperature on MHC class IIβ amino acid diversity of the peptide-binding region (PBR) in Atlantic salmon populations. The three best-fit models are presented and the best model is in bold. The number of parameters (*k*), the AIC_C values corrected for small sample size, the relative differences in AIC_C values (ΔAIC_C) and the relative weight (ωAIC_C) are indicated for each model.

Best-fit Model	K	AIC_C	ΔAIC_C	ωAIC_C
Bacteria	**2**	**−48.1**	**0**	**0.559**
Temperature	2	−47.0	1.1	0.323
Bacteria ∗ Temperature	2	−44.5	3.6	0.092

Discussion

Numerous empirical studies have confirmed that selection is acting on MHC variation (reviewed in Bernatchez and Landry 2003; Piertney and Oliver 2006). Only a handful of studies, however, have allowed apportioning in wild populations the effects of selection and neutral evolutionary forces (Cohen 2002; Westerdahl et al. 2004; van Oosterhout et al. 2006; Eyto et al. 2007). Moreover, studies on natural populations have not specifically identified which factors may underpin selection at MHC genes (but see Prugnolle et al. 2005 on human MHC) (Piertney and Oliver 2006). Given both empirical and theoretical evidence of an association between pathogen diversity and virulence with temperature (Griffiths 1991; Larsen et al. 2004; Zheng et al. 2004), our objective was to test the hypothesis that the strength of balancing selection imposed by pathogens should vary with temperature and result in a temperature gradient of MHC diversity in Atlantic salmon. The present results reveal that MHC class IIβ allelic diversity increased with temperature and resulted in a latitudinal gradient, in contrast with the general pattern observed at neutral microsatellites. More importantly, our results also showed that MHC amino acid diversity increased with temperature at the pathogen-binding region (PBR) but not at the non-PBR. This analysis also allowed us to identify three amino acid sites that were largely responsible for the observed relationship between MHC diversity and temperature. Finally, the analyses of bacterial diversity in rivers revealed a positive relationship between bacterial and MHC diversity that was more pronounced for PBR than for non-PBR. Bacterial diversity was also an important factor retained by the model selection approach for explaining MHC amino acid diversity at PBR for the subset of rivers analyzed. Overall, this study identified temperature as a potential selective agent influencing MHC diversity in wild populations. Based on the bacterial diversity results and given the putative role of temperature in shaping large-scale patterns of pathogen diversity and virulence, our results support the hypothesis that selection associated with pathogens via a temperature gradient plays a major role in shaping MHC diversity in Atlantic salmon over most of its North American distribution range.

Before firmly concluding that the pattern we observed does indeed provide evidence for variation in balancing selection associated with a temperature gradient, we must consider the possibility that factors other than selection can also produce clinal variation in MHC diversity with temperature along a latitudinal gradient. Effective population size (N_e) is theoretically expected to influence levels of neutral genetic diversity maintained in wild populations, a pattern that has been reported in many empirical studies on overexploited or endangered species of fish (Hauser et al. 2002; Knaepkens et al. 2004). Here, the possibility that the pattern of MHC diversity is mainly driven by the effective population size was not supported because no relationship was observed between effective population size and MHC diversity and no particular spatial organization of N_e was observed. Population genetic diversity may also result from colonization history and/or restricted gene flow between populations. In North America, Atlantic salmon colonization is hypothesized to have occurred from south to north from a southern Atlantic glacial refugium located off the New England coast (Schmidt 1986). It has also been documented that this colonization process was progressive for salmonids and occurred stepwise along the North American Coast (Castric and Bernatchez 2003). In this context, each colonization step toward the north could be realized by a smaller and less genetically diverse group of colonizers, which could reduce genetic diversity at neutral markers on a clinal gradient from south to north. In such a case, one would expect to observe an overall increase in allele diversity with temperature for both MHC and microsatellites, a pattern that was not well supported by our results. Indeed, even though two individual loci did not conform to the general trend, the diversity pattern depicted for microsatellites did not show an overall association with temperature along the latitudinal gradient in this system. This suggests that the probable colonization process did not result in a global loss of genetic diversity at neutral markers, in contrast with the pattern observed at MHC. Moreover, under the scenario in which colonization and contemporary gene flow would have a strong impact on MHC diversity, we could also expect that a pattern of IBD would prevail for both MHC and microsatellite markers, as previously observed at microsatellites for other fishes in North America (Turgeon and Bernatchez 2001; Castric and Bernatchez 2003; Olsen et al. 2003). This pattern was not supported by our results. Indeed, only microsatellites showed an IBD pattern, which suggests that colonization history and contemporary gene flow have played a more predominant role in shaping genetic diversity at neutral markers than at MHC. Overall, the comparison between MHC and microsatellite markers better supports the alternative hypothesis that selection associated with

temperature has an important role in shaping MHC allelic diversity in Atlantic salmon populations along a latitudinal gradient.

The contrast between MHC and neutral molecular sites is accentuated when considering the amino acid level, which underlines the importance of using the genetic level most closely related to selection when studying local adaptation. Indeed, MHC alleles cannot necessarily be translated directly into immune defence diversity as some alleles may function similarly in terms of immune defence by encoding proteins that bind similar peptide repertoires. In this study, significant differences in amino acid diversity patterns were observed between the PBR compared to other sites of the MHC class II. As in most other studies on salmonids (Miller and Withler 1996; Landry and Bernatchez 2001), we found that the ratio of nonsynonymous to synonymous substitutions (*dn*/*ds*) was much higher than unity for PBR but not for non-PBR, confirming that positive selection is acting specifically on the pathogen-binding region. Most importantly, PBR diversity strongly increased with temperature, an association that was not found at non-PBR. This supports the contention that selection associated with temperature was responsible for shaping diversity at the pathogen-binding region. To our knowledge, the comparison of the relationship between PBR and non-PBR with a putative selective agent has never been reported for MHC genes in wild populations. Based on both allelic and amino acid results at MHC, we are unable to identify a possible selective agent other than temperature that could be confounded with the observed temperature–MHC diversity relationship. We propose that the selective effect imposed by temperature represents the most plausible and parsimonious explanation associated with the MHC diversity pattern documented in this study.

Although temperature has been identified as an important selective agent in this study, its influence on MHC diversity may be indirect. Based on theoretical evidence suggesting that pathogen diversity, virulence, and exposure increase with temperature (Rohde and Heap 1998; Guernier et al. 2004; Larsen et al. 2004; André and Day 2005), pathogen-driven balancing selection is a plausible mechanism influencing MHC diversity across a temperature gradient. Our results on bacterial diversity in rivers are consistent with this hypothesis. Although we did not document bacterial diversity on a wide temperature scale and in all rivers, a significant increase in MHC diversity with bacterial diversity was observed. This association was more pronounced at PBR than at other sites. Moreover, bacterial diversity was the principal factor explaining PBR variability in a model selection approach, although a model with temperature was also adequate. This suggests that, over a narrow temperature scale, bacterial diversity could have a predominant role in shaping MHC amino acid diversity in wild populations of Atlantic salmon. A previous study suggested that parasites in the environment might be tightly linked to eutrophication and pollution and such toxic components might have a direct or indirect effect on the MHC profile (Cohen 2002). In our system, water quality of the rivers analyzed for bacterial diversity is classified as good to very good (class A) based on nine water quality indexes (coliform bacteria, chlorophyll a, nitrogen, nitrites and nitrates, phosphorus, organic carbon, suspended solids, pH, and turbidity) evaluated through the watershed monitoring program of the Québec provincial government since 1979 (http://www.mddep.gouv.qc.ca/eau/inter˙en.htm). As such, water quality is most likely not a potential mechanism explaining the observed relationship between bacterial diversity and MHC variability in this system. Although we cannot rule out the possibility that other factors may influence the relationship observed here, we suggest that changes in the intensity of pathogen-driven balancing selection along a temperature gradient is a likely mechanism explaining the association between MHC diversity and temperature. The nature of the association between MHC amino acid diversity at PBR and both temperature and bacterial diversity is best explained by a logarithmic equation. MHC diversity increases rapidly at relatively low temperature and low bacterial diversity levels and seems to increase slowly thereafter. This could suggest that MHC amino acid diversity in Atlantic salmon is approaching a maximum plateau in this system. Indeed, as suggested by a recent experimental analysis on viruses, the fitness of a population adapting to a specific environment does not increase indefinitely, but instead reaches a plateau due to the intrinsic nature of its finite population and to the dynamics of the mutational effects (Silander et al. 2007). However, the type of association along with the possible underlying causes must be considered with caution at this point. Although the association between bacterial diversity and MHC variability at PBR is clearly shown, future studies could detail that association by measuring pathogen diversity on a broader temperature scale to achieve more power. An association between pathogen and MHC diversity at a large spatial scale has also been observed in a recent study on human populations. Diversity at HLA (MHC for human) class I was correlated with the richness of intracellular human disease agents, and microsatellites were used to dissociate the effect of selection from human colonization history processes (Prugnolle et al. 2005). Results of this study supported the pathogen-driven balancing selection hypothesis and suggested that changes in pathogen richness have been an important selective force maintaining HLA diversity in humans. Based on these and our results, we propose that temperature influences pathogen diversity and virulence and creates a clinal change in pathogen-driven balancing selection intensity, which in turns influences host local adaptation and results in a diversity gradient at MHC genes. In this context, it is also plausible to hypothesize that directional selection may gradually replace balancing selection in extreme environments in which only one or few pathogens can survive for a specific host. This could explain partly the evidence for directional selection observed in some populations of sockeye

salmon at MHC (Miller et al. 2001) and the low MHC diversity found in some aquatic vertebrates (Towsdale et al. 1989; Slade 1992; Weber et al. 2004). When considering the documented influence of temperature on bacterial diversity and virulence, it is likely that the selective force identified in this study could have an important impact on local adaptation of other organisms. As a consequence, and in a climate change context, we believe that this research avenue represents a priority in conservation genetics.

In conclusion, this study aimed at contributing to elucidate the evolutionary basis of MHC diversity in wild populations of Atlantic salmon. Our results are consistent with the hypothesis that MHC diversity is largely driven by variation in balancing selection intensity associated with a temperature gradient, being higher in warm than in cold environments. Although more research is needed to test the hypothesis, we propose that changes in pathogen-driven balancing selection could be responsible for shaping MHC diversity with temperature. Overall, this study differentiated between the influence of natural selection and other evolutionary forces, identified temperature as a selective agent and provided support for the role of pathogens as a selective pressure influencing MHC diversity at a large spatial scale in the wild. As such, this study represents an example of local adaptation and complements a handful of studies that have identified a selective agent explaining MHC variation in natural populations.

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Supplementary Material

The following supplementary material is available for this article:

Appendix S1. Genetic diversity and sample statistics for MHC class IIβ and microsatellite loci.
Appendix S2. Methods.
Appendix Literature Cited.

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