

# Putative causes and consequences of MHC variation within and between locally adapted stickleback demes

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## Abstract

Genes of the major histocompatibility complex (MHC) have been a source of considerable research interest, owing in large part to the growing body of evidence that they may be subject to both natural and sexual selection. However, much remains to be learned about the dynamics of MHC genes in subdivided populations, particularly those characterized by divergent ecological pressures. In this study, we attempt to disentangle the relative roles of both parasite-mediated selection and MHC-mediated mate choice in an open estuarine system inhabited by two parapatric, adaptively divergent threespine stickleback (*Gasterosteus aculeatus*) demes. We sequenced the putative peptide-binding region (PBR) of an estimated four Class II $\beta$  loci from 127 individuals, identifying 329 sequence variants (276 translated amino acid sequences). Demes differed significantly both in the frequency of MHC alleles and in the communities of helminth parasites infecting resident sticklebacks. Strong signatures of natural selection were inferred from analyses of codon substitutions, particularly in the derived (freshwater) rather than the ancestral (marine) deme. Relationships between parasite load and MHC diversity were indicative of balancing selection, but only within the freshwater deme. Signals of MHC-mediated mate choice were weak and differed significantly between demes. Moreover, MHC-mediated mate choice was significantly influenced by environmental salinity and appeared of secondary importance to tendencies towards assortative mating. We discuss the implications of these findings in respect to ecological adaptation and the potential demographic consequences of possible outcomes of MHC-mediated mate choice.

**Keywords:** assortative mating, balancing selection, *Gasterosteus aculeatus*, mate choice, parasites

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## Introduction

Genes of the major histocompatibility complex (MHC) are the most polymorphic known to vertebrates (Hughes & Yeager 1998; Bernatchez & Landry 2003; Klein *et al.* 2007), and it is largely believed that selective pressures imposed by pathogens are responsible for maintaining diversity at these loci (Parham & Ohta 1996; Jeffery & Bangham 2000; Klein *et al.* 2007). Indeed, many valuable insights into the nature of bal-

ancing selection can be traced to the study of MHC polymorphisms, particularly in wild, outbred populations (Ilmonen *et al.* 2007). Examples of MHC heterozygote advantage are often reported from studies of wild populations (Richman *et al.* 2001; Evans & Neff 2009; Kekäläinen *et al.* 2009), even in those of small effective size, potentially prone to loss of diversity through random processes (van Oosterhout *et al.* 2006). Alternatively, frequency dependence has been invoked as the dominant mechanism underlying balancing selection on MHC genes (Schierup *et al.* 2000; Borghans *et al.* 2004), and though it has been difficult to document conclusively, a few well-studied examples are known (Paterson *et al.* 1998; Westerdahl *et al.* 2004). Many other reported examples of specific MHC alleles conferring

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resistance to a unique pathogen challenge may also be indicative of frequency-dependent selection (Lanfords *et al.* 2001; Froeschke & Sommer 2005; Harf & Sommer 2005; Schou *et al.* 2007; Croisetière *et al.* 2008; Lenz *et al.* 2009).

Sexual selection can play an equally important role in the maintenance of MHC polymorphisms (Penn 2002), although as in the case of balancing selection, debate remains as to the dominant role of two competing mechanisms: selection for maximal vs. optimal diversity. Interestingly, this is also germane to the distinction between good- and compatible-genes models of indirect fitness benefits accrued through mate choice based upon assessment of a potential partner's genetic quality (Neff & Pitcher 2005). There is considerable evidence to suggest that MHC gene products may facilitate olfactory recognition, and mate choice to maximize MHC diversity has been proposed as a mechanism for inbreeding avoidance (reviewed in Penn & Potts 1999; Penn 2002; Bernatchez & Landry 2003; Consuegra & Garcia de Leaniz 2008; Setchell *et al.* 2010), though this strategy may be equally prominent independent of relatedness (Landry *et al.* 2001; Neff *et al.* 2008). Alternatively and more intuitively appealing as an explanation for MHC polymorphism are observations of a preference for sexual partners with an intermediate level of MHC diversity (Wedekind & Furi 1997; Forsberg *et al.* 2007). This notion has been extended to species with multiple copies of MHC paralogues in which a self-referencing strategy may be used to choose a mate most likely to produce offspring with an optimal number of unique alleles (Aeschlimann *et al.* 2003; Bonneaud *et al.* 2006).

Yet the dual effects of balancing and sexual selection may have contradictory demographic and evolutionary consequences. For example, in the absence of physical barriers between locally adapted demes, female mate choice to increase MHC distance could favour disassortative mating, which in turn would promote admixture to the detriment of locally adapted genes. Balancing selection in subdivided populations might also serve to promote gene flow: while overdominant selection may favour high genetic diversity within demes, a gene invading from another may be quickly selected for (Schierup *et al.* 2000). Consequently, given the range of selective forces potentially acting upon MHC genes, and the inconsistency in their outcomes, there is much to be learned in even the best-studied model systems.

In recent years, the threespine stickleback (*Gasterosteus aculeatus*) has emerged as an important model for MHC studies. Its genome contains at least four known MHC class II paralogues located on two distinct chromosomes (Sato *et al.* 2000; Reusch *et al.* 2004; Reusch & Lanfords 2005). Maintenance of MHC

polymorphism has largely been attributed to ecological factors. Nonlinear correlations between individual allelic richness and macroparasite infestation suggested that MHC diversity may be under balancing selection (Wegner *et al.* 2003b), and experimental results have largely supported this inference (Wegner *et al.* 2003a; Kurtz *et al.* 2004). MHC heterozygosity in sticklebacks is also negatively correlated with MHC gene expression (Wegner *et al.* 2006), and high levels of MHC expression are linked to poor body condition and elevated oxidative stress (Kurtz *et al.* 2006). Consequently, fitness is likely maximized in individuals with an optimal level of MHC diversity. Moreover, reproductive success also appears to be greatest in individuals with an optimal allele number (Kalbe *et al.* 2009).

The St. Lawrence River estuary presents an appealing ecosystem to address the role of MHC polymorphism within the context of adaptive divergence. Sticklebacks are indigenous to the system and are partitioned into two demes whose geographic ranges coincide with the ecological division into freshwater and saltwater zones (McCairns & Bernatchez 2008); moreover, salinity differences represent unique selective pressures driving adaptive divergence between demes (McCairns & Bernatchez 2010). The estuary is a large, open waterway, free of physical barriers to dispersal, although gene flow across the dominant ecological gradient is likely reduced via decreased survival of larval hybrids (McCairns & Bernatchez 2010). Although divergent behavioural preferences leading to reproductive isolation have evolved between resident freshwater and anadromous Japanese populations (Ishikawa & Mori 2000; Kitano *et al.* 2007), it is unknown if prezygotic barriers to admixture exist within the St. Lawrence. Knowledge of MHC variation within this system is also lacking, as is any information on related selective pressures.

In this study, we document natural levels of MHC variation, both within and between demes, and test for associated signals of natural selection, particularly in relation to anticipated divergent parasite infestations. At the same time, we evaluate the relative importance of sexual selection acting via MHC-mediated mate choice and infer the potential demographic consequences of MHC-mediated reproductive behaviour. To address this issue, however, requires consideration of the predominant environmental variation between demes. For example, translocation experiments have shown that common, habitat-specific environmental effects influence odour cues used by sticklebacks in shoaling behaviour (Ward *et al.* 2007; Webster *et al.* 2007). Water chemistry can also affect olfactory recognition (Heuschele & Candolin 2007). Consequently, to minimize bias in tests between demes originating from divergent

physicochemical conditions, experiments are performed under salinity conditions typical for both demes.

## Methods

We sampled fish using seine nets, dip nets and minnow traps from two spawning/nursery sites (CR & FOR), each located within the geographic range of distinct, locally adapted demes (McCairns & Bernatchez 2008, see also Fig. S1, Supporting information). We collected only sexually mature fish of the same size/age class. Fish were transported alive to wet laboratory facilities (LARSA, Université Laval) and randomly assigned to one of two salinities representative of natural conditions, either saltwater (20‰) or freshwater (<1‰). Thus, all fish were acclimated to test salinities prior to mating trials. Males were housed separately in individual aquaria to prevent aggressive interactions, whereas females were kept in groups of approximately 20 fish per aquarium. Fish were fed *ad libitum* twice daily a mixture of flake food and commercial salmonid fry ration, in addition to once daily supplements of freeze-dried *Mysis relicta*, frozen chironomid larvae and live *Artemia* nauplii. Photoperiod mimicked natural conditions, consisting of a 30-min 'sunrise' in which luminosity increased gradually to ambient (40%) levels, was maintained for 15 h, then followed by a 30-min 'sunset' and 8-h darkness.

### Mate choice experiments

Mate choice experiments were based on 36 experimental trials, 18 in freshwater and 18 in saltwater. A total of 18 individual females from each deme were used, nine in each of the two environments. Twelve independent 500-L tanks were isolated in a quiet, screened corner of the wet laboratory. Six tanks were filled with artificial seawater, and the remaining six filled with dechlorinated freshwater. Tanks measured 200 cm × 50 cm × 50 cm. Each was partitioned into two end sections (50 cm × 50 cm and 75 cm in length) and a middle section (50 cm × 50 cm × 50 cm). Dividers were porous and consisted of two components, both an opaque and a transparent layer, which could be removed independently. Natural nesting materials (i.e. fine aquatic vegetation and filamentous algae) and a 30-cm-diameter plastic dish filled with fine-grained sand were placed into opposing corners in each end. Plates were oriented cross-diagonally and bordered by artificial vegetation to limit visual contact between nesting males.

As mate size is a predominant trait attractive to stickleback females (Cubillos & Guderley 2000; Ishikawa & Mori 2000; Albert 2005; Boughman *et al.* 2005; Ólafsdóttir *et al.* 2006), males were carefully size-matched within

each trial. Two equal sized males, one from each deme, were selected for transfer to spawning tanks. One male, selected at random, was marked with a small clip to the second dorsal spine. Both males were then assigned randomly to opposite ends of a spawning tank. Males were allowed 72 h to construct a nest or were replaced: only actively nesting males were used in tests. Following nest construction, a randomly selected female was transferred to the centre section of each tank. The female was allowed to acclimate for 24 h, during which time she had only olfactory contact with males. Opaque barriers were subsequently removed for a period of 20 min, permitting both olfactory and visual contact with males. Physical barriers were then removed, and the female was allowed to mate with the male of her choice. After removal of barriers, fish were neither observed nor disturbed, apart from daily feeding.

After 4 days, fish were removed from the spawning tanks. A barrier was placed in the centre of each tank to restrict movement. Fish were removed with a dip net, and males were identified by inspecting the spine clip to verify that they had remained associated with their respective nests. Nests were removed and all eggs counted. All fish were euthanized, individually bagged and identified and flash frozen for subsequent necropsy. Tanks were then completely drained, rinsed and re-filled with new water for two additional series of experiments.

### Parasitology

Fish used in mating trials were thawed individually, then weighed and measured prior to necropsy ( $n = 105$ ; one trial group removed because of female mortality). Gills were excised whole, sectioned by arches and halved for ease of mount. Gill sections were inspected for parasitic copepods and monogenean trematodes under a dissecting microscope. Next, an incision was made longitudinally along the entire ventral surface. All fins, membranes and body tissues were inspected for metacercariae of digenean trematodes (blackspot). In the case of females, remaining ovaries/eggs were weighed, then dissected for the removal of nematodes. Testes were pressed between glass slides and examined microscopically for the presence of nematodes. Viscera were carefully separated, and any helminths found free or attached to mesenteries were removed and enumerated. The liver was pressed between glass slides and inspected under magnification, for ease of enumerating encysted larval cestodes (plerocercoids). Finally, both the stomach and intestine were sectioned longitudinally, washed with distilled water and inspected under magnification for helminths. Additionally, stomach/intestinal contents were screened for helminths. Parasites were

identified to broad taxonomic groups, corresponding to Class or Order and separated by either adult or larval stage. As a simple description of differences between demes, each taxon was analysed separately: count data were treated as dependent variables, and differences between demes determined by generalized linear models using log-link functions and incorporating quasi-Poisson distributed errors, to compensate for overdispersion.

### MHC genotyping

MHC genotyping was accomplished in multiple stages including gene amplification, allele separation by cloning, preliminary screening of clones and finally Sanger sequencing. In total, 127 individuals were genotyped. These included all fish used in the mate choice experiments, in addition to unused samples collected from spawning sites, totalling 63 individuals from the freshwater deme and 64 from saltwater.

DNA was obtained from fin tissues via Protease K digestion followed by salt extraction (Aljanabi & Martinez 1997). We selected a forward (CAG CAG CTC AGT GGG GAA G) and reverse (GTG GTT CAG ACA GTA AAC CTC CTT C) primer designed to amplify multiple copies of MHC class II $\beta$  loci, specifically within the putative peptide-binding region (PBR) of each paralogue (Reusch *et al.* 2004). A BLAST search for the primer sequences within the stickleback genome indicated 100% identity of the forward primer with all annotated MHC paralogues, and >90% sequence identity between the reverse primer and three of four annotated MHC paralogues. The remaining annotated gene copy shared only 60% sequence identity; however, its 3' terminus, corresponding to the primer annealing site, contained an 8 bp GC-rich sequence, which facilitated 'mismatch' annealing by reducing annealing temperature and adjusting reaction MgCl<sub>2</sub> concentrations. PCR conditions are detailed in Table S1 (Supporting information).

PCR products were purified by electrophoresis on 2% agarose gel and extracted with a QIAquick Gel Extraction kit (QIAGEN, Valencia, CA, USA). Purified amplicons were inserted into TopoTA plasmid vectors and competent *Escherichia coli* cells following manufacturer's protocols (Invitrogen, Carlsbad, CA, USA). Bacterial colonies were grown on antibiotic infused agar plates, incubated at 37 °C for 16 h. Sixteen positive colonies per individual were selected at random. Plasmid DNA was obtained by heat extraction in TBE buffer, then used as template for PCR using the same MHC primers, though with a 6-FAM-labelled forward primer. Sequence variants were identified by single-strand conformation polymorphism (SSCP), run in capillary electrophoresis on an ABI3100 genetic analyzer

(Applied Biosystems, Carlsbad, CA, USA). Electropherograms were scored and binned using GENEMARKER software (SoftGenetics, State College, PA, USA). SSCP screening was performed on an individual basis, in which all 16 cloned haplotypes of a given individual were scored and binned simultaneously, and samples of plasmid DNA corresponding to any unique intra-individual migration variants were targeted for sequencing.

Dye terminator sequencing was performed on an ABI3100 genetic analyzer (Plate-forme d'Analyses Biomoléculaires, Université Laval). Sequencing reactions used the same MHC primers, and each clone was sequenced using both forward and reverse primers. Manual verification/correction of electropherograms, in addition to alignment of forward and reverse sequences, was performed manually using PROSEQ (Filatov 2002). In total, we identified and sequenced 600 plasmids, including re-runs for ambiguous electropherograms. We also re-sequenced an additional 40 plasmids, selected at random, to estimate error because of PCR artefacts. Primer sequences were excluded from the final assembled contigs, which were translated into amino acid sequences, herein referred to as PBR sequences, matching those first detailed in the literature (Reusch *et al.* 2004).

### Data analyses

Given the known MHC duplication within *Gasterosteus aculeatus* (Sato *et al.* 1998; Reusch *et al.* 2004) and that four annotated MHC genes exhibited high sequence similarity with the primers used in this study, we expected heterozygous individuals to possess up to a maximum of eight unique MHC alleles/haplotypes (hereafter simply referred to as alleles). As fish were sampled randomly from spawning sites, we estimated the frequency of copy-number heterozygotes in each deme based on the distribution observed in samples. Confidence limits for these estimates were obtained by nonparametric bootstrapping (10 000 iterations). Intra-individual MHC diversity was estimated by calculating the average pairwise protein distance based on PBR amino acid sequences. Protein distances were based on the Dayhoff PAM matrix (Dayhoff *et al.* 1979), calculated using Felsenstein's (2005) PROTDIST program, called from within BIOEDIT (Hall 1999). For each class of copy-number heterozygote, we estimate mean MHC differentiation, also based on estimated amino acid distances, with confidence limits from nonparametric bootstrapping (10 000 iterations). Finally, we tested for MHC differentiation between demes by calculating  $F_{ST}$  based on haplotype frequencies, evaluated by 1000 permutations, using ARLEQUIN (Excoffier *et al.* 2005). Additionally, we estimated the degree of divergence between demes using both  $G'_{ST}$

(Hedrick 2005) and Jost's (2008) index of divergence ( $D_J$ ), calculated using SMOGD (Crawford 2010). These were contrasted with estimates of divergence based on nine microsatellite loci, from data described in a previous study (McCairns & Bernatchez 2008).

We tested for signatures of selection within sequence data, based on the ratio of nonsynonymous (dN) to synonymous (dS) substitutions at all codons. Given that estimates of dN and/or dS can be biased when based on short nucleotide sequences (Nei & Kumar 2000), we performed bootstrapped comparisons of differences directly on data corresponding to the proportion of the respective substitutions at each site (p-distance), averaged over all sequence pairs, using the Nei–Gojobori algorithm implemented in MEGA4 (Tamura *et al.* 2007). We performed analyses on the entire data set and on subsets of sequences common and private to each deme. To compliment these data, we also tested for associations between degree of parasite infestation and intra-individual MHC diversity. All statistical modelling was performed in the R computing language (R Development Core Team 2007). We estimated the degree of parasite infestation as the total parasite load, irrespective of taxon, and by an index of parasite diversity, the Shannon–Wiener index (H). We modelled both linear and quadratic relationships simultaneously for two independent variables describing MHC diversity: the number of unique PBR sequences for a given individual, and the average, intra-individual pairwise protein distance. Relationships with parasite load were evaluated from generalized linear models (quasi-Poisson errors; log-link functions). Parasite diversity data conformed to assumptions of normality and homoscedasticity; therefore, relationships with MHC diversity were evaluated by general linear models with Gaussian error.

We tested for the evidence of assortative mating and/or environmental effects on female reproductive choice by modelling the probability of male reproductive success as a function of deme of origin of both pairs in a potential coupling and the environment in which the mating occurred (i.e. native or foreign to the female). Additionally, to ascertain whether MHC genes might be implicated in mate choice, and thus also subject to sexual selective pressures, we tested for associations between natural levels of MHC variation and reproductive success. Reproductive success for each male was assigned as a binary variable dependent upon whether they were selected as a mate by the female in their respective test. As an initial predictor, we considered the number of unique PBR sequences of each male. However, previous observations suggest that MHC-mediated mate choice decisions may be made via self-reference mechanisms (Reusch *et al.* 2001; Aeschlimann *et al.* 2003). Consequently, we considered two addi-

tional predictor variables. The first was a count of the total number of unique PBR sequences between members of a potential reproductive couple. The second metric was an average of all pairwise protein distances between male and female PBR sequences. Finally, we sought to combine insight regarding MHC-mediated mate choice with tests of assortative mating. In all cases, linear and quadratic relationships with MHC variability, in addition to interactive effects with spawning environment, were evaluated by generalized linear models incorporating binomial errors and logit-link functions. Raw data files have been deposited in the Dryad data repository (DOI: 10.5061/dryad.7926).

## Results

### MHC variation

Forty randomly chosen clones sequenced twice possessed identical reads, suggesting that experimental error was less than 1/40 (0.025). Of 600 haplotypes sequenced (both strands), we identified 325 unique 127 bp DNA sequences and 4 unique indels (126 bp). Only 39 sequences were common to both demes, with 143 (102 singletons) private alleles assigned to the freshwater deme and 147 (111 singletons) private to the maritime deme (Table 1; GenBank accession numbers HQ418503–HQ418831). A BLAST search of *Gasterosteus* entries in GenBank annotated as MHC Class II $\beta$ -chain sequences yielded a total of 84 entries, all emanating from western European populations. After trimming regions outside of the amplicon range used in this study, 58 unique European sequences were retained for comparison. Only 11 of these 58 were identical to the 329 sequences we detected: three were common to both demes, five were private to the freshwater deme, and three were private to the saltwater deme. Thus, in total, we have identified 318 novel MHC sequences from the St. Lawrence estuary. An unrooted phylogeny of these 376 MHC Class II sequences (47 European; 329 St. Lawrence estuary) is available as supplementary information (Fig. S2, Supporting information).

The 329 DNA sequences identified from the estuary corresponded to 276 unique PBR sequences, after translation. Thirty-nine PBR sequences were common to both demes, whereas 114 were private to the freshwater deme (FW) and 123 private to the maritime deme (SW). Given the large number of private alleles, PBR sequence frequency was low in each deme (FW = 0–3.6%; SW = 0–4.2%). Phylogenetic analysis of the 276 amino acid sequences revealed no significant deme-specific PBR clades (Fig. S3, Supporting information).

Individual variation ranged from one to seven unique PBR sequences. Although 24 individuals were identified

**Table 1** Codon-based tests of neutral evolution averaged over sequence pairs. Analyses are based on the Nei–Gojobori method, as implemented in MEGA4; standard error (SE) estimates are from 1000 bootstrapping iterations. The number of sequences and the total number of sites (in parentheses) used in each analysis are presented in the first column (*N*). Estimates of average evolutionary divergence are based on the number of nonsynonymous differences per nonsynonymous sites (dN) and the proportional number of synonymous differences (dS)

Sequences	<i>N</i>	dN (SE)	dS (SE)	dN/dS	<i>P</i> -value
All	329 (21)	0.170 (0.040)	0.060 (0.020)	3.323	0.001
Shared	39 (26)	0.148 (0.035)	0.073 (0.036)	1.593	0.114
FW (all)	182 (21)	0.170 (0.038)	0.059 (0.020)	3.152	0.002
FW (private)	143 (21)	0.172 (0.039)	0.066 (0.022)	2.916	0.004
SW (all)	186 (24)	0.159 (0.035)	0.079 (0.031)	1.749	0.083
SW (private)	147 (24)	0.161 (0.037)	0.085 (0.036)	1.655	0.101

FW, freshwater deme; SW, maritime deme.

with eight unique MHC alleles, none possessed more than seven unique PBR sequences. The majority of individuals from each deme could be described as intermediate copy-number heterozygotes: 69.8% of FW individuals and 65.4% of SW individuals had between three and five unique PBR sequences (Fig. 1a). Moreover, homozygotes (1.9% FW; 3.8% SW) and heterozygotes possessing more than five alleles (3.8% FW; 5.8% SW) were relatively infrequent in both demes. Intra-individual variation among PBR sequences did not differ between demes (Fig. 1b). Analysis of allele frequencies indicated a weak, yet significant, differentiation between demes ( $F_{ST} = 0.001$ ;  $P = 0.034$ ). Indices of population divergence based on MHC allele frequencies ( $G'_{ST} = 0.043$ ;  $D_J = 0.041$ ) also revealed differentiation between demes, but less so than for estimates averaged over nine microsatellite loci ( $G'_{ST} = 0.081$ ;  $D_J = 0.078$ ), based on McCairns & Bernatchez (2008).

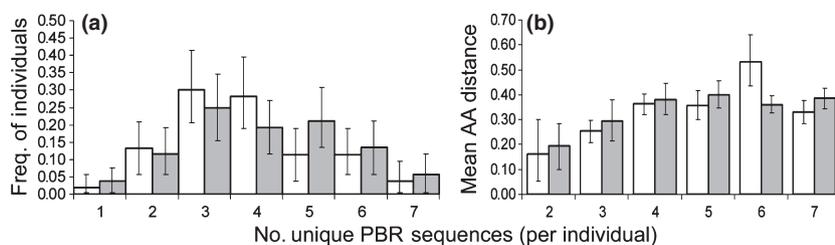
### Signatures of selection

Averaged over the putative PBR coding sequence for all haplotypes, we detected a significantly greater proportion of nonsynonymous substitutions among codon sites (Table 1); however, the 39 alleles shared between

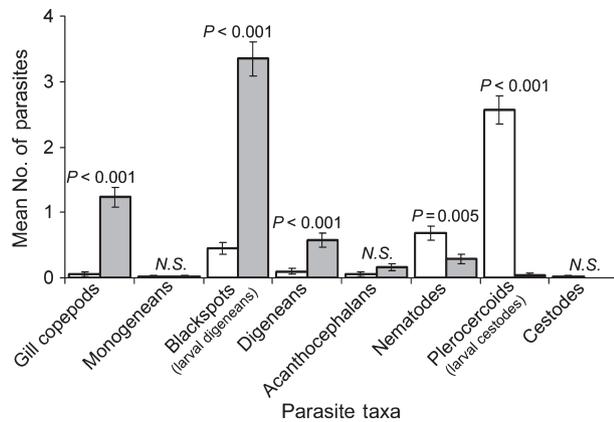
demes showed no signature of selection. Data sets containing only haplotypes found in the FW deme exhibited significantly higher nonsynonymous than synonymous substitutions. Conversely, sequence data from the SW deme showed no evidence of selection.

Fish sampled from each deme were infested by different parasite taxa (Fig. 2). Ectoparasitic copepods, subcutaneously encysted trematode metacercariae (blackspots), and digenean trematodes were significantly more prevalent in SW individuals. Freshwater fish carried greater loads of nematodes and larval cestodes found primarily encysted in the liver. Total parasite load did not differ between demes (Table 2), although maritime (SW) fish were infested with a greater diversity of parasite taxa (Fig. 3; Table S2, Supporting information). And although spine clipping can stimulate an immune response leading to the clearing of some parasite taxa (Wedekind & Little 2004), preliminary analyses revealed no such difference between males used in this experiment (Table S3, Supporting information).

Relationships between individual MHC diversity and degree of parasitic infestation differed between demes. Analysis of parasite load revealed a significant interaction effect between PBR number and deme of origin (Table 2). Separate analysis within the freshwater deme



**Fig. 1** Estimated frequency distribution of individuals with multiple, variant MHC sequences (a) and the average intra-individual pairwise amino acid distance for each class of copy-number heterozygote (b). Data are based on translated amino acid sequences containing the putative peptide-binding region (PBR) for MHC Class II $\beta$  loci. Protein distances are based on the Dayhoff PAM matrix, calculated for each pairwise combination within each individual. Freshwater individuals are denoted by white bars; grey bars correspond to the maritime deme. Error bars represent 95% confidence limits for the parameter estimates, obtained by nonparametric bootstrapping (10 000 iterations). PBR, peptide-binding region; MHC, major histocompatibility complex.



**Fig. 2** Average intensities of infestation for various parasite taxa in fish sampled from the freshwater (FW; white bars) and maritime (SW; grey bars) demes. Plots are based on the mean number of a given taxa within individuals from each deme  $\pm$  standard errors of the estimates. *P*-values above each taxon bar describe the significance of the difference between demes; N.S. denotes not significant ( $\alpha = 0.05$ ).

indicated a significant quadratic relationship (Table 2; Fig. 3a). In contrast, we observed no significant effect of PBR number on parasite load within the maritime deme (Table 2; Fig. 3a). Intra-individual MHC variation, based on average pairwise protein distances of translated PBR sequences, exhibited no significant association with parasite load, nor difference between demes (Table 2; Fig. 3b). Similar analyses of parasite diversity revealed no significant association with either metric of MHC diversity (Figs 3c,d; Table S2, Supporting information).

Joint analyses of both metrics of MHC diversity indicated significant interaction effects between PBR number and average intra-individual protein distance associated with total parasite load (Table 2), but not with parasite diversity (Table S2, Supporting information). Analysis of parasite load also indicated a significant three-way interaction with deme ( $P = 0.011$ ). Deme-specific analyses revealed that the interaction between metrics of MHC diversity and parasite load was significant only in the freshwater deme (Table 2). Response contours describing this effect suggest an optimal level of overall MHC diversity within the freshwater deme: reduced parasite load was associated with either increased differentiation of few PBR sequences or greater numbers of less divergent PBR sequences (Fig. 4).

#### MHC and reproductive success

Neither spine cutting nor parasite load influenced a male's reproductive success (Table S3, Supporting information), and so in the interest of parsimony, was deemed inconsequential for subsequent analyses. All

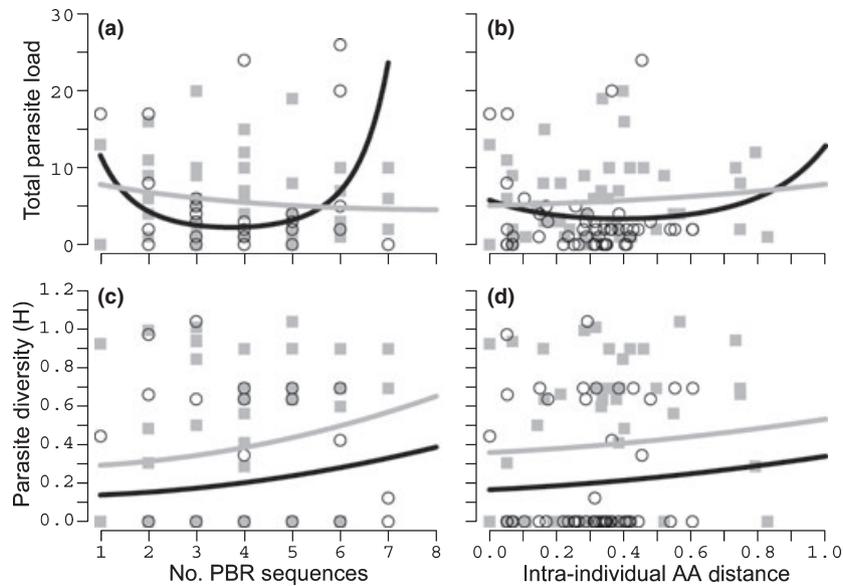
analyses of MHC-mediated mate choice were complicated by significant three-way interactions between deme, spawning environment and the various metrics of MHC diversity. Consequently, data specific to each deme were treated independently, although these analyses were also complicated by interactions with test environment. Within a female's native salinity, number of male PBR sequences was not significantly associated with reproductive success (Table 3). Only maritime (SW) females in freshwater exhibited a significant preference for males with an intermediate number of PBR sequences (Fig. 5a). Total number of unique PBR sequences within a potential couple was unrelated to reproductive success in maritime females, irrespective of spawning environment (Table 3). FW females in their native environment appeared to favour couplings, which yielded an intermediate number of total PBR sequences, although analysis of these data separately suggested the relationship was not strictly significant ( $P = 0.068$ ). This effect was inverted in the foreign salinity, with females apparently disfavouring pairings that would yield an intermediate number of PBR ( $P = 0.030$ ; Fig. 5b). Trends in male reproductive success data suggested that females from both demes, when in their native environments (solid lines; Fig. 5c), appeared to favour males with similar PBR sequences; however, this relationship was not statistically significant (Table 3). In contrast, males with an intermediate degree of MHC differentiation appeared to be favoured by all females, when in their foreign environment.

Simple tests of assortative mating were equivocal. Maritime females exhibited a significant overall preference for males originating from the same deme ( $P = 0.019$ ), independent of both environmental ( $P = 0.256$ ) and interaction ( $P = 0.108$ ) effects. Analysis of freshwater female mate choice revealed a nearly significant interaction effect between origin of male and spawning environment ( $P = 0.066$ ), although further exploration of these data separated by environment suggested no clear male preference in either the native freshwater ( $P = 0.323$ ) or the foreign environment ( $P = 0.121$ ). However, joint analysis of FW female mate choice cues revealed a significant preference for males from the same deme, although this preference appears to be disrupted when tests are conducted in a foreign environment (Table 4, Fig. 6). When in their native salinity, freshwater females exhibited a self-referential strategy of mate choice, as evidenced by MHC compliments of mating pairs, which would yield a putatively optimal number of PBR diversity in their offspring (see Figs 3a and 5b). However, homodemic males were preferred over heterodemic males with a similar MHC complement and also preferred over a greater range of MHC complements (Fig. 6a).

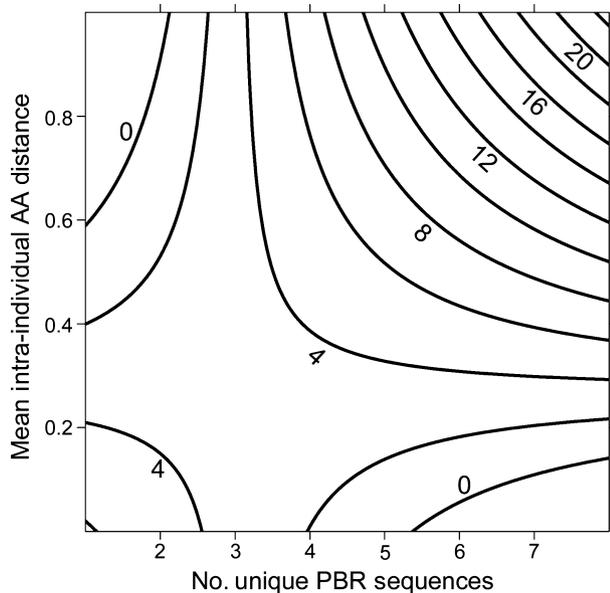
**Table 2** Analysis of deviance for models describing linear and quadratic relationships between two metrics of individual MHC diversity, the number of unique PBR sequences or the average intra-individual protein (AA) distance and total parasite load. A model including the interaction between MHC metrics is also presented. *P*-values define the significance of the respective model terms in reducing model deviance, corrected for overdispersion by incorporating quasi-Poisson distributed errors. Full models describe both simple, additive differences and interaction effects between demes. Simple effects of MHC diversity are also modelled separately for each deme. Graphical interpretations of these models are presented in Figs 3 and 4

Model terms	Full model				Freshwater deme (FW)				Maritime deme (SW)						
	d.f.	Deviance	Residual d.f.	Residual deviance	<i>P</i> -value	d.f.	Deviance	Residual d.f.	Residual deviance	<i>P</i> -value	d.f.	Deviance	Residual d.f.	Residual deviance	<i>P</i> -value
NULL			104	679.27	—			52	445.71	—			51	223.00	—
No. PBR sequences	1	8.27	103	671.00	0.260	1	48.56	51	397.15	0.017	1	5.28	50	217.71	0.276
Quadratic (PBR)	1	55.58	102	615.42	0.004	1	85.05	50	312.11	0.002	1	0.20	49	217.51	0.831
Deme	1	6.16	101	609.25	0.331										
PBR × deme	1	33.30	100	575.96	0.024										
Quadratic (PBR) × deme	1	46.34	99	529.62	0.008										
NULL			104	679.27	—			52	445.71	—			51	223.00	—
Average AA distance	1	19.81	103	659.46	0.143	1	23.06	51	422.66	0.196	1	2.24	50	220.76	0.485
Quadratic (AA)	1	27.17	102	632.29	0.086	1	35.15	50	387.50	0.110	1	0.33	49	220.43	0.789
Deme	1	12.34	101	619.95	0.248										
AA × deme	1	0.04	100	619.91	0.945										
Quadratic (AA) × deme	1	11.97	99	607.93	0.255										
NULL			104	679.27	—			52	445.71	—			51	223.00	—
No. PBR sequences	1	8.27	103	671.00	0.280	1	48.56	51	397.15	0.025	1	5.28	50	217.17	0.274
Average AA distance	1	13.09	102	657.91	0.174	1	3.56	50	393.59	0.545	1	5.95	49	211.76	0.245
Deme	1	9.05	101	648.86	0.259	—	—	—	—	—	—	—	—	—	—
No. PBR × AA distance	1	30.92	100	617.95	0.036	1	74.70	49	318.89	0.006	1	1.57	48	210.19	0.551
No. PBR × deme	1	31.65	99	586.30	0.035										
AA distance × deme	1	11.10	98	575.20	0.211										
PBR × AA × deme	1	46.12	97	529.08	0.011										

PBR, peptide-binding region; MHC, major histocompatibility complex.



**Fig. 3** Relationships between metrics of parasite infestation and individual MHC diversity (see Table 2 and Table S2, Supporting information). Fish from the freshwater deme (FW) are represented by black lines and open circles; grey lines and solid squares correspond to the maritime deme (SW). MHC diversity is estimated as either the number of unique PBR sequences for a given individual or the average pairwise protein (AA) distance within an individual. PBR, peptide-binding region; MHC, major histocompatibility complex.



**Fig. 4** Response contours describing total parasite load in fish from the freshwater deme (FW) as a function of the interaction between intra-individual PBR number and their average protein (AA) distance (see Table 2). PBR, peptide-binding region.

**Discussion**

Two striking observations emerge from these results, both related to the remarkable patterns of MHC diversity detected in this system. First, the number of MHC

alleles observed is substantially greater than that reported from other wild stickleback populations. Second, signatures at the molecular level provide strong evidence of positive selection. This begs the question how such a high level of polymorphism can be maintained in the face of directional selection between demes. There is in fact mounting empirical evidence that despite balancing selection-maintaining polymorphism within populations, spatially divergent selective pressures can produce discrete variation between demes (Ekblom *et al.* 2007; Alcaide *et al.* 2008). Detailed phylogenetic analyses of closely related, sympatric African cichlids have demonstrated that balancing selection can maintain a high level of diversity at MHC loci, while divergent, parasite-mediated selection can simultaneously promote differentiation between congeners (Blais *et al.* 2007). We contend that observations of St. Lawrence sticklebacks show similar patterns below the species level.

*Divergent selection between demes*

Geographic patterns in MHC diversity from many wild populations are suggestive of adaptation to localized selective pressures (Bowen *et al.* 2006; Dionne *et al.* 2007, 2009; Ekblom *et al.* 2007). Within the St. Lawrence estuary, MHC allele frequencies differ significantly between demes, although the level of divergence is weak ( $F_{ST} = 0.001$ ), perhaps owing to the small sample

**Table 3** Analysis of deviance for models describing linear and quadratic relationships between MHC diversity and female mate choice in both native and foreign salinities. Significant three-way interactions between MHC diversity, environment and deme (see Results) necessitated analysis of demes separately. Models include the number of unique PBR sequences of potential mates (Fig. 5a); the total number of combined, unique PBR sequences per potential couple (Fig. 5b); and the average pairwise protein (AA) distance between male and female PBR sequences of a potential couple (Fig. 5c)

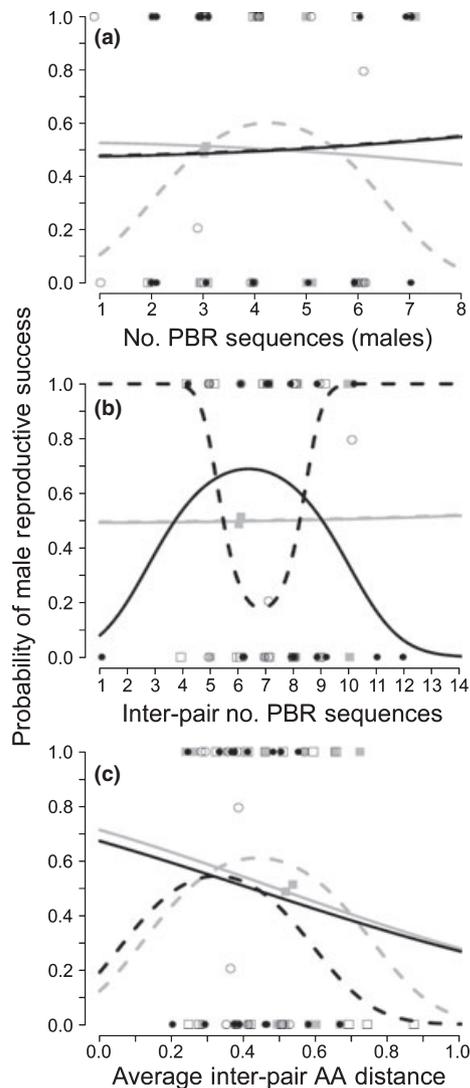
Model terms	FW females					SW females				
	d.f.	Deviance	Residual d.f.	Residual deviance	P-value	d.f.	Deviance	Residual d.f.	Residual deviance	P-value
NULL			29	41.589	—			27	38.816	—
No. PBR sequences	1	0.010	28	41.579	0.921	1	0.000	26	38.816	>0.999
Quadratic (PBR)	1	0.400	27	41.179	0.527	1	1.370	25	37.446	0.242
Environment	1	0.005	26	41.175	0.946	1	0.044	24	37.403	0.835
PBR × environment	1	0.620	25	40.555	0.431	1	0.451	23	36.952	0.502
Quadratic (PBR) × environment	1	0.191	24	40.363	0.662	1	6.222	22	30.730	0.013
NULL			29	41.589	—			27	38.816	—
Inter-pair no. PBR	1	0.065	28	41.524	0.799	1	0.000	26	38.816	>0.999
Quadratic (PBR)	1	1.311	27	40.213	0.252	1	0.055	25	38.761	0.814
Environment	1	0.149	26	40.065	0.700	1	0.000	24	38.761	0.992
PBR × environment	1	0.234	25	39.831	0.629	1	0.572	23	38.189	0.449
Quadratic (PBR) × environment	1	6.307	24	33.524	0.012	1	0.000	22	38.188	0.984
NULL			29	41.589	—			27	38.816	—
Inter-pair AA distance	1	0.715	28	40.874	0.398	1	0.571	26	38.246	0.450
Quadratic (AA)	1	0.275	27	40.599	0.600	1	0.831	25	37.414	0.362
Environment	1	0.042	26	40.557	0.837	1	0.025	24	37.390	0.875
AA × environment	1	0.324	25	40.233	0.569	1	0.154	23	37.236	0.695
Quadratic (AA) × environment	1	5.435	24	34.798	0.020	1	6.507	22	30.729	0.011

PBR, peptide-binding region; MHC, major histocompatibility complex; FW, freshwater deme; SW, maritime deme.

size relative to the number of observed alleles, or because of limitations when  $F_{ST}$  is calculated from highly diverse marker sets (Hedrick 2005). Although frequency-based estimates of MHC divergence alone should be viewed with some scepticism (Bernatchez & Landry 2003), comparisons of synonymous and nonsynonymous substitutions provide rather compelling evidence that MHC evolution has not been because of strictly neutral processes (Table 1). Moreover, differences observed between demes are suggestive of divergent selective pressures. The dN/dS ratio averaged over all alleles (3.323) is both highly significant and similar in magnitude to that observed in comparisons across species of wild primates (Garamszegi *et al.* 2009) and ungulates (Schaschl *et al.* 2006). Additionally, this ratio falls within the range of predicted values from simulations of divergent lineages under differing selection pressures (Kryazhimskiy & Plotkin 2008). Equally telling are the discrepancies observed between demes. Within the presumed ancestral lineage (SW), the dN/dS ratio does not differ significantly from unity, whereas in the derived deme inhabiting the novel freshwater environment (FW), a clear signal of positive selection is observed (Table 1). Such divergent molecular signals are consistent with divergent selection. It should be

noted, however, that this inference is contingent upon the assumption that none of the MHC copies analysed are pseudogenes, an unlikely scenario given that evidence from expression studies suggest that all gene copies are functional (Kurtz *et al.* 2006; Wegner *et al.* 2006; Scharsack *et al.* 2007b).

Necropsy results can also be argued to corroborate the molecular inference of divergent selection. Clear differences exist in the types of parasite taxa infecting individuals from the respective demes, and relationships between MHC diversity and parasite infestation also differ between them. In the maritime deme, only a small proportion of variation in parasite load can be explained by metrics of MHC diversity; however, within the derived, freshwater deme, a significant proportion of model deviance is explained by these variables (Table 2). Of course this line of reasoning is based on the premise that parasite load represents a fitness cost. In sticklebacks, macroparasite infection is associated with increased expression of MHC genes thereby implying parasite infestation can represent a sufficient stress to warrant mounting an energetically costly immune response (Wegner *et al.* 2006). Additionally, experiments in semi-natural field enclosures have revealed that interactive effects between environmental



**Fig. 5** Relationships between different metrics of MHC diversity/differentiation and the probability of reproductive success. Environment-dependant female mate choice, as determined from generalized linear models (Table 3), is described by the respective curves. Freshwater females (FW) are plotted in black, whereas females from the maritime deme (SW) are plotted in grey. Solid lines represent test results within a female's native salinity (i.e. <math><1\text{‰}</math> for FW; <math>20\text{‰}</math> for SW), whereas dashed lines correspond to results within the foreign environment (i.e. <math><1\text{‰}</math> for SW; <math>20\text{‰}</math> for FW). Models include the number of unique PBR sequences of potential mates (a); the total number of combined, unique PBR sequences per potential couple (b); and the average pairwise protein (AA) distance between male and female PBR sequences of a potential couple (c). PBR, peptide-binding region; MHC, major histocompatibility complex.

stress and parasite load can have dramatic fitness consequences (Wegner *et al.* 2008). Moreover, previous work within the SW deme of the St. Lawrence system revealed an effect of parasite load on reproductive success (Blais *et al.* 2004). Unfortunately, experiments con-

trolling for divergent MHC background and habitat effects have been inconclusive regarding genotype-by-environment ( $G \times E$ ) interactions for parasite resistance (Rauch *et al.* 2006), though this may depend upon which parasite taxa are considered (Kalbe & Kurtz 2006). Certainly, evidence from divergent lake-river stickleback populations suggest that adaptation to localized parasite taxa may be typical for the species (Scharsack *et al.* 2007a).

#### Maintenance of polymorphism within demes

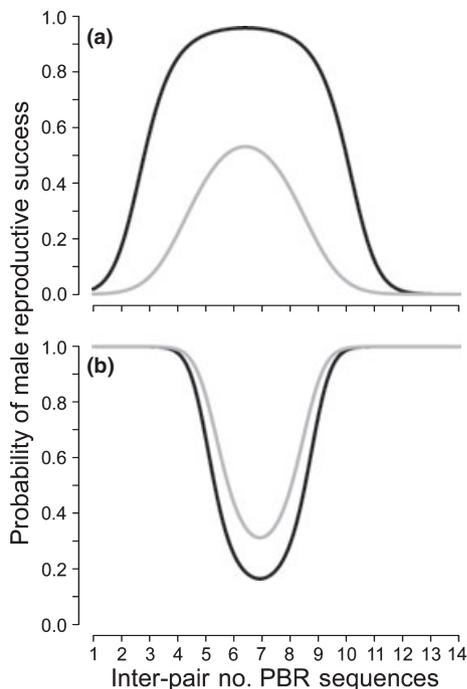
Although variants cannot be assigned to specific loci, overall polymorphism observed in the present study is comparable to the vast diversity described for the human MHC gene, *DRB1* (Klein *et al.* 2007). Such hypervariability is essentially the norm for MHC loci in most vertebrate species (see Introduction), and at the time of writing, a search for orthologous sequences within GenBank revealed substantial levels of variation in other teleost fishes (e.g. *Onchorhynchus mykiss*,  $n = 250$ ; *Salmo salar*,  $n = 199$ ; *Danio rerio*,  $n = 184$ ). Yet, despite considerable research into stickleback MHC variation over the past decade, only 84 unique *Gasterosteus aculeatus* paralogues had been reported in GenBank. The identification of 329 unique sequences in this study exceeded expectations and represents a substantially greater genetic diversity than that reported from other wild, outbred populations (Wegner *et al.* 2003b). This is unlikely due to PCR and/or cloning and sequencing error. First, both strands were sequenced, and both revealed the same sequences. Also, none of the 40 replicate sequences differed from their first read, indicating that actual experimental error was likely less than 1/40 (0.025). At this error rate, only 15 (i.e.  $0.025 \times 600$ ) of 329 identified sequences would potentially be false positives. Consequently, our sample, though likely not exhaustive, should represent an unbiased estimation of naturally occurring variation.

Many lines of evidence suggest that the high degree of polymorphism observed in this system is maintained by some form of balancing selection. Divergence estimators are nearly twice as great for microsatellites than when based on MHC data, and  $F_{ST}$  estimates from MHC haplotype frequencies are 6 $\times$  less than those based on neutral markers (see McCairns & Bernatchez 2008), both observations which correspond to predictions of balancing selection (Muirhead 2001). Additionally, clusters of MHC haplotypes are characterized by shallow branch lengths (Fig. S2, Supporting information), a pattern concordant with simulations of MHC diversity generated by overdominant selection (van Oosterhout 2009). Implicit within the overdominance hypothesis is the assumption that heterozygosity is

**Table 4** Analysis of deviance for models describing linear and quadratic relationships between MHC diversity and mate choice, relative to assortative or disassortative mating, for females originating from the freshwater (FW) deme. Models include the total number of combined, unique PBR sequences per potential couple in the native, freshwater environment (Fig. 6a) and the foreign, saltwater (SW) environment (Fig. 6b)

Model terms	FW environment					SW environment				
	d.f.	Deviance	Residual d.f.	Residual deviance	P-value	d.f.	Deviance	Residual d.f.	Residual deviance	P-value
NULL			15	22.181	—			13	19.408	—
Inter-pair no. PBR	1	0.149	14	22.032	0.700	1	0.033	12	19.375	0.856
Quadratic (PBR)	1	3.189	13	18.842	0.074	1	4.693	11	14.682	0.030
Male origin (deme)	1	3.955	12	14.887	0.047	1	0.312	10	14.370	0.577
PBR × deme	1	0.171	11	14.716	0.679	1	1.168	9	13.202	0.280
Quadratic (PBR) × deme	1	2.193	10	12.524	0.139	1	1.109	8	12.094	0.292

PBR, peptide-binding region; MHC, major histocompatibility complex.



**Fig. 6** Probability of assortative (homodemic) vs. disassortative (heterodemic) mating for females originating from the freshwater deme (FW), relative to the relationships between the total number of combined, unique PBR sequences per potential couple. Average reproductive success of homodemic (i.e. FW) males is plotted in black, whereas grey lines denote the reproductive success of heterodemic (i.e. SW) males. Environment-dependant female mate choice is determined from separate generalized linear models (Table 4) corresponding to females observed in their native (i.e. freshwater; a) and foreign (i.e. saltwater; b) environments. PBR, peptide-binding region.

favourable, irrespective of which alleles are present (Takahata & Nei 1990; De Boer *et al.* 2004). For MHC, this is hypothesized to be because of degenerate binding sites capable of recognizing a wide variety of anti-

gen types (Parham & Ohta 1996; Klein *et al.* 2007). Consequently, favourable alleles could be replaced by almost any new MHC mutant, which in turn would increase the allelic turnover rate and reduce the maximum persistence time of alleles (van Oosterhout 2009), a scenario which could also explain the large number of singletons observed. Conversely, the abundance of private alleles occurring at low frequencies is also consistent with simulations of host–pathogen co-evolutionary dynamics under frequency-dependent selection (Borghans *et al.* 2004), although under this model a high specificity between particular PBR sequences and co-evolving pathogen antigens is implicit. And although we cannot distinguish between the relative importance of heterozygote advantage vs. negative frequency-dependent selection, this may be moot given that the two need not be mutually exclusive (Parham & Ohta 1996; Froeschke & Sommer 2005; Spurgin & Richardson 2010).

The negative quadratic relationship between parasite load and MHC diversity in the freshwater deme is consistent with models of balancing selection and also concordant with observations from other stickleback systems in which fitness is maximized via optimal, rather than maximum, MHC heterozygosity (Wegner *et al.* 2003a,b; Kurtz *et al.* 2004). This optimality may reflect a potentially detrimental, hyperactive autoimmune response that can result when MHC genes are excessively diverse (Nowak *et al.* 1992; Mason 2001). Interestingly, the interaction between metrics of MHC diversity suggest a possible trade-off between allele number and differentiation, wherein parasite load is reduced in individuals with an ‘optimal’ MHC diversity defined either via few alleles with a large AA distance, or via a greater number of similar alleles (Fig. 4). This trade-off highlights an alternative mechanism by which organisms with multiple MHC copies might attain an

optimal level of genetic diversity, although it should be noted that this hypothesis is predicated on the assumption that AA distance is a potentially meaningful index in *Gasterosteus*, as reported previously for Atlantic salmon (Landry *et al.* 2001). Given the emerging perspective that intra-individual MHC variation may be critical in the positive selection phase of maintaining standing T-cell receptor variation (reviewed in Woelfing *et al.* 2009), this would seem to be an avenue worth exploring in more detail for future studies.

In contrast to the largely congruent evidence for natural selection acting upon MHC loci, data pertaining to the role of sexual selection could best be described as ambiguous. Moreover, many of the observations emanating from this experiment conflict with earlier work from independent stickleback populations. For example, with the exception of males courting maritime females in their foreign salinity (i.e. freshwater), male heterozygosity was unrelated to reproductive success (Fig. 5a; Table 3). This is in stark contrast to earlier results, suggesting that females prefer males with an intermediate number of MHC alleles (Reusch *et al.* 2001; Aeschlimann *et al.* 2003; Milinski *et al.* 2005). Further consideration of a potential self-referencing strategy, in which females are thought to prefer males with a 'complimentary' number of MHC alleles to produce offspring with an intermediate number of alleles, yielded equally equivocal results. This strategy was entirely nonexistent in all maritime females, whereas only freshwater females within their native osmotic environment exhibited a tendency towards mate choice decisions leading to 'optimally' heterozygous offspring (Figs 5b and 6a). Moreover, this relationship was reversed when individuals from this deme were exposed to their foreign environment. Consequently, we are left to question the universality of MHC-mediated mate choice within the species.

#### *Is MHC a universal mate choice signal?*

Female sticklebacks are attracted by olfactory cues emanating from MHC ligands (Milinski *et al.* 2005). Use of olfactory signals are presumed to play a role in mate choice given that female sticklebacks prefer the odour of males with a greater number of PBR copies (Reusch *et al.* 2001), although actual mate choice decisions may be somewhat more complex, wherein females use a self-referencing strategy to attain an optimal allele count in their offspring (Aeschlimann *et al.* 2003). The most convincing examples of MHC-mediated mate choice in sticklebacks have been based on female preferences inferred only from behavioural cues, not realized reproduction (Reusch *et al.* 2001; Aeschlimann *et al.* 2003; Milinski *et al.* 2005). While this approach may be bene-

ficial in terms of separating the role of MHC from other traits, it cannot be forgotten that actual mate choice is multimodal (Blais *et al.* 2004) and that absence of complete information can potentially bias outcomes (Nilsson & Nilsson 2000; McLennan 2003). This is not to suggest that our work is perfect in this regard. For example, nuptial colouration has been shown to influence female mate choice (Kraak & Bakker 1998; Cubillos & Guderley 2000; McLennan 2003), although not in all populations (Braithwaite & Barber 2000; Scott 2004). Unfortunately, we could not directly measure male colour, although all males exhibited typical nuptial colouration. Nevertheless, any subtle differences in male colour may also be captured in MHC diversity, given that male colouration has been demonstrated to be an honest signal of both genetic and paternal quality (Candolin 2000; Barber *et al.* 2001; Jäger *et al.* 2007).

What is most apparent from our observations is a substantive environmental effect. In all models, relationships between mate choice and metrics of MHC diversity exhibited significant deme  $\times$  environment interactions, suggesting not only differences between demes in the importance of MHC-mediated mate choice, but that any odour-based cues may be influenced or disrupted by environmental salinity. Social recognition and preferential shoaling behaviour in sticklebacks are known to be influenced by shared environmental salinity (Ward *et al.* 2007). Recent tests have also revealed significant environment-dependent differences in the relative importance of odour-based vs. visual mate choice cues (Heuschele *et al.* 2009). We suggest that observed salinity effects may represent an important environmental influence on odour and/or mate quality perception. Such environmental, context-dependent mate choice has not been lost on behavioural ecologists (Jennions & Petrie 1997; Qvarnström 2001), and it is certainly relevant to stickleback biology. Our explicit testing of this context dependence has revealed a potentially important environmental sensitivity to what has been assumed as a dominant mate choice cue. We contend that consideration of such context-dependent behaviour is essential, particularly for inferences regarding the potential for gene flow in other open systems wherein intra-specific divergence is driven by physicochemical properties of the aquatic environment.

This insight is perhaps best exemplified through consideration of the freshwater deme. From an evolutionary perspective, this represents a derived subpopulation adapted to a novel osmoregulatory environment (McCairns & Bernatchez 2010). Consequently, it may be most deleteriously affected by gene flow from neighbouring populations. Females from this deme were the only group to exhibit self-referential, MHC-mediated mating preferences (Fig. 5b; Table 3), but no apparent

assortative mating behaviour. However, when these relationships were considered in concert, a very different perspective emerged: heterodemic couplings were favoured only when such pairings yield the strictest conditions of optimality (i.e. offspring with the potential for 3–4 unique PBR alleles), although assortative mating was more probable given a homodemic partner with a similar allelic compliment (Fig. 6a). Moreover, a greater degree of putatively nonoptimal allelic combinations appeared to be tolerated in favour of assortative mating (Fig. 6a; Table 4). However, if the differential environmental conditions of potential heterodemic couplings had not been considered, we could have easily interpreted random or even disassortative mating (Fig. 6b).

## Conclusions

Natural selection, potentially driven by divergent parasite communities, appears to be the dominant evolutionary force, which has helped to shape extant patterns of MHC diversity, both within and between stickleback demes of the St. Lawrence estuary. Patterns in molecular data and relationships with parasites lend support to the conclusion that high levels of MHC variation are maintained by balancing selection operating differentially within each deme. Conversely, it is unclear what role, if any, sexual selection may play in maintaining this polymorphism. Given natural levels of MHC variation, there is little evidence to suggest that mate choice decisions are reached primarily by assessment of a potential mate's MHC compliment. Significant differences exist between demes in the relationship between MHC diversity and reproductive success. Moreover, the role of any MHC-mediated cues appears to be context-dependent and significantly influenced by environmental variation. We contend that in the light of context dependence, MHC-mediated mate choice may be of secondary importance to factors such as assortative mating. Thus, while MHC assessment of mate quality can be important, it is almost certainly not a universal cue in reproductive systems with active mate choice (Pateron & Pemberton 1997; Westerdahl 2004). Moreover, our results highlight that population and/or environmental considerations should inspire pause for thought prior to future tests of mate choice strategies.

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## References

- Aeschlimann PB, Häberli MA, Reusch TBH, Boehm T, Milinski M (2003) Female sticklebacks *Gasterosteus aculeatus* use self-reference to optimize MHC allele number during mate selection. *Behavioral Ecology and Sociobiology*, **54**, 119–126.
- Albert AYK (2005) Mate choice, sexual imprinting, and speciation: a test of a one-allele isolating mechanism in sympatric sticklebacks. *Evolution*, **59**, 927–931.
- Alcaide M, Edwards SV, Negro JJ, Serrano D, Tella JL (2008) Extensive polymorphism and geographical variation at a positively selected MHC class IIB gene of the lesser kestrel (*Falco naumanni*). *Molecular Ecology*, **17**, 2652–2665.
- Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, **25**, 4692–4693.
- Barber I, Arnott SA, Braithwaite VA, Andrew J, Huntingford FA (2001) Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 71–76.
- Bernatchez L, Landry C (2003) MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? *Journal of Evolutionary Biology*, **16**, 363–377.
- Blais J, Rico C, Bernatchez L (2004) Nonlinear effects of female mate choice in wild threespine sticklebacks. *Evolution*, **58**, 2498–2510.
- Blais J, Rico C, van Oosterhout C, Cable J, Turner GF, Bernatchez L (2007) MHC adaptive divergence between closely related and sympatric African cichlids. *PLoS ONE*, **2**, e734.
- Bonneaud C, Chastel O, Federici P, Westerdahl H, Sorci G (2006) Complex MHC-based mate choice in a wild passerine. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 1111–1116.
- Borghans JAM, Beltman JB, De Boer RJ (2004) MHC polymorphism under host–pathogen coevolution. *Immunogenetics*, **55**, 732–739.
- Boughman JW, Rundle HD, Schluter D (2005) Parallel evolution of sexual isolation in sticklebacks. *Evolution*, **59**, 361–373.
- Bowen L, Aldridge BM, DeLong R *et al.* (2006) MHC gene configuration variation in geographically disparate populations of California sea lions (*Zalophus californianus*). *Molecular Ecology*, **15**, 529–533.
- Braithwaite VA, Barber I (2000) Limitations to colour-based sexual preferences in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology*, **47**, 413–416.
- Candolin U (2000) Male-male competition ensures honest signaling of male parental ability in the three-spined

- stickleback (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology*, **49**, 57–61.
- Consuegra S, Garcia de Leaniz C (2008) MHC-mediated mate choice increases parasite resistance in salmon. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 1397–1403.
- Crawford NG (2010) SMOGD: software for the measurement of genetic diversity. *Molecular Ecology Resources*, **10**, 556–557.
- Croiseti re S, Tarte PD, Bernatchez L, Belhumeur P (2008) Identification of MHC class II beta resistance/susceptibility alleles to *Aeromonas salmonicida* in brook charr (*Salvelinus fontinalis*). *Molecular Immunology*, **45**, 3107–3116.
- Cubillos ER, Guderley HE (2000) Analysis of the factors related with mate choice and reproductive success in male three-spined sticklebacks. *Journal of Fish Biology*, **56**, 1201–1216.
- Dayhoff MO, Schwartz RM, Orcutt BC (1979) A model of evolutionary change in proteins. In: *Atlas of Protein Sequence and Structure* (ed Dayhoff MO). pp. 345–352. National Biomedical Research Foundation, Silver Spring, MD.
- De Boer RJ, Borghans JAM, van Boven M, Kesmir C, Weissing FJ (2004) Heterozygote advantage fails to explain the high degree of polymorphism of the MHC. *Immunogenetics*, **55**, 725–731.
- Dionne M, Miller KM, Dodson JJ, Caron F, Bernatchez L (2007) Clinal variation in MHC diversity with temperature: evidence for the role of host–pathogen interaction on local adaptation in Atlantic salmon. *Evolution*, **61**, 2154–2164.
- Dionne M, Miller KM, Dodson JJ, Bernatchez L (2009) MHC standing genetic variation and pathogen resistance in wild Atlantic salmon. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **364**, 1555–1565.
- Eklom R, Saether SA, Jacobsson P *et al.* (2007) Spatial pattern of MHC class II variation in the great snipe (*Gallinago media*). *Molecular Ecology*, **16**, 1439–1451.
- Evans ML, Neff BD (2009) Major histocompatibility complex heterozygote advantage and widespread bacterial infections in populations of Chinook salmon (*Oncorhynchus tshawytscha*). *Molecular Ecology*, **18**, 4716–4729.
- Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.
- Felsenstein J (2005) *PHYMLIP (Phylogeny Inference Package) Version 3.6*. Distributed by the author, Department of Genome Sciences, University of Washington, Seattle, WA.
- Filatov DA (2002) PROSEQ: a software for preparation and evolutionary analysis of DNA sequence data sets. *Molecular Ecology Notes*, **2**, 621–624.
- Forsberg LA, Dannowitz J, Petersson E, Grahn M (2007) Influence of genetic dissimilarity in the reproductive success and mate choice of brown trout: females fishing for optimal MHC dissimilarity. *Journal of Evolutionary Biology*, **20**, 1859–1869.
- Froeschke G, Sommer S (2005) MHC class II DRB variability and parasite load in the striped mouse (*Rhabdomys pumilio*) in the southern Kalahari. *Molecular Biology and Evolution*, **22**, 1254–1259.
- Garamszegi LZ, de Groot NG, Bontrop RE (2009) Correlated evolution of nucleotide substitution rates and allelic variation in MHC-DRB lineages of primates. *BMC Evolutionary Biology*, **9**, doi: 10.1186/1471-2148-9-73.
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95–98.
- Harf R, Sommer S (2005) Association between major histocompatibility complex class II DRB alleles and parasite load in the hairy-footed gerbil, *Gerbillurus paeba*, in the southern Kalahari. *Molecular Ecology*, **14**, 85–91.
- Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution*, **59**, 1633–1638.
- Heuschele J, Candolin U (2007) An increase in pH boosts olfactory communication in sticklebacks. *Biology Letters*, **3**, 411–413.
- Heuschele J, Mannerla M, Gienapp P, Candolin U (2009) Environment-dependent use of mate choice cues in sticklebacks. *Behavioral Ecology*, **20**, 1223–1227.
- Hughes AL, Yeager M (1998) Natural selection at major histocompatibility complex loci of vertebrates. *Annual Review of Genetics*, **32**, 415–435.
- Ilmonen P, Penn DJ, Damjanovich K, Morrison L, Ghotbi L, Potts WK (2007) Major histocompatibility complex heterozygosity reduces fitness in experimentally infected mice. *Genetics*, **176**, 2501–2508.
- Ishikawa M, Mori S (2000) Mating success and male courtship behaviors in three populations of the threespine stickleback. *Behaviour*, **137**, 1065–1080.
- J ager I, Eizaguirre C, Griffiths SW *et al.* (2007) Individual MHC class I and MHC class IIB diversities are associated with male and female reproductive traits in the three-spined stickleback. *Journal of Evolutionary Biology*, **20**, 2005–2015.
- Jeffery KJM, Bangham CRM (2000) Do infectious diseases drive MHC diversity? *Microbes and Infection*, **2**, 1335–1341.
- Jennions MD, Petrie M (1997) Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews of the Cambridge Philosophical Society*, **72**, 283–327.
- Just L (2008)  $G_{ST}$  and its relatives do not measure differentiation. *Molecular Ecology*, **17**, 4015–4026.
- Kalbe M, Kurtz J (2006) Local differences in immunocompetence reflect resistance of sticklebacks against the eye fluke *Diplostomum pseudospathaceum*. *Parasitology*, **132**, 105–116.
- Kalbe M, Eizaguirre C, Dankert I *et al.* (2009) Lifetime reproductive success is maximized with optimal major histocompatibility complex diversity. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 925–934.
- Kek al inen J, Vallunen JA, Primmer CR, R atty  J, Taskinen J (2009) Signals of major histocompatibility complex overdominance in a wild salmonid population. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 3133–3140.
- Kitano J, Mori S, Peichel CL (2007) Phenotypic divergence and reproductive isolation between sympatric forms of Japanese threespine sticklebacks. *Biological Journal of the Linnean Society*, **91**, 671–685.
- Klein J, Sato A, Nikolaidis N (2007) MHC, TSP, and the origin of species: from immunogenetics to evolutionary genetics. *Annual Review of Genetics*, **41**, 281–304.
- Kraak SBM, Bakker TCM (1998) Mutual mate choice in sticklebacks: attractive males choose big females, which lay big eggs. *Animal Behaviour*, **56**, 859–866.
- Kryazhimskiy S, Plotkin JB (2008) The population genetics of dN/dS. *PLoS Genetics*, **4**, e1000304.

- Kurtz J, Kalbe M, Aeschlimann PB *et al.* (2004) Major histocompatibility complex diversity influences parasite resistance and innate immunity in sticklebacks. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **271**, 197–204.
- Kurtz J, Wegner KM, Kalbe M *et al.* (2006) MHC genes and oxidative stress in sticklebacks: an immuno-ecological approach. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 1407–1414.
- Landry C, Garant D, Duchesne P, Bernatchez L (2001) 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 1279–1285.
- Langefors Å, Lohm J, Grahn M, Andersen O, von Schantz T (2001) Association between major histocompatibility complex class IIB alleles and resistance to *Aeromonas salmonicida* in Atlantic salmon. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 479–485.
- Lenz TL, Eizaguirre C, Scharsack JP, Kalbe M, Milinski M (2009) Disentangling the role of MHC-dependent 'good genes' and 'compatible genes' in mate-choice decisions of three-spined sticklebacks *Gasterosteus aculeatus* under semi-natural conditions. *Journal of Fish Biology*, **75**, 2122–2142.
- Mason D (2001) Some quantitative aspects of T-cell repertoire selection: the requirement for regulatory T cells. *Immunological Reviews*, **182**, 80–88.
- McCairns RJS, Bernatchez L (2008) Landscape genetic analyses reveal cryptic population structure and putative selection gradients in a large-scale estuarine environment. *Molecular Ecology*, **17**, 3901–3916.
- McCairns RJS, Bernatchez L (2010) Adaptive divergence between freshwater and marine sticklebacks: insights into the role of phenotypic plasticity from an integrated analysis of candidate gene expression. *Evolution*, **64**, 1029–1047.
- McLennan DA (2003) The importance of olfactory signals in the gasterosteid mating system: sticklebacks go multimodal. *Biological Journal of the Linnean Society*, **80**, 555–572.
- Milinski M, Griffiths S, Wegner KM, Reusch TBH, Haas-Assenbaum A, Boehm T (2005) Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 4414–4418.
- Muirhead CA (2001) Consequences of population structure on genes under balancing selection. *Evolution*, **55**, 1532–1541.
- Neff BD, Pitcher TE (2005) Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. *Molecular Ecology*, **14**, 19–38.
- Neff BD, Garner SR, Heath JW, Heath D (2008) The MHC and non-random mating in a captive population of chinook salmon. *Heredity*, **101**, 175–185.
- Nei M, Kumar S (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, NY.
- Nilsson SO, Nilsson GE (2000) Free choice by female sticklebacks: lack of preference for male dominance traits. *Canadian Journal of Zoology*, **78**, 1251–1258.
- Nowak MA, Tarczyhorno K, Austyn JM (1992) The optimal number of major histocompatibility complex molecules in an individual. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 10896–10899.
- Ólafsdóttir GÁ, Ritchie MG, Snorrason SS (2006) Positive assortative mating between recently described sympatric morphs of Icelandic sticklebacks. *Biology Letters*, **2**, 250–252.
- van Oosterhout C (2009) A new theory of MHC evolution: beyond selection on the immune genes. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 657–665.
- van Oosterhout C, Joyce DA, Cummings SM *et al.* (2006) Balancing selection, random genetic drift, and genetic variation at the major histocompatibility complex in two wild populations of guppies (*Poecilia reticulata*). *Evolution*, **60**, 2562–2574.
- Parham P, Ohta T (1996) Population biology of antigen presentation by MHC class I molecules. *Science*, **272**, 67–74.
- Paterson S, Pemberton JM (1997) No evidence for major histocompatibility complex-dependent mating patterns in a free-living ruminant population. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **264**, 1813–1819.
- Paterson S, Wilson K, Pemberton JM (1998) Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 3714–3719.
- Penn DJ (2002) The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology*, **108**, 1–21.
- Penn DJ, Potts WK (1999) The evolution of mating preferences and major histocompatibility complex genes. *American Naturalist*, **153**, 145–164.
- Qvarnström A (2001) Context-dependent genetic benefits from mate choice. *Trends in Ecology & Evolution*, **16**, 5–7.
- R Development Core Team (2007) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rauch G, Kalbe M, Reusch TBH (2006) Relative importance of MHC and genetic background for parasite load in a field experiment. *Evolutionary Ecology Research*, **8**, 373–386.
- Reusch TBH, Langefors Å (2005) Inter- and intralocus recombination drive MHC class IIB gene diversification in a teleost, the three-spined stickleback *Gasterosteus aculeatus*. *Journal of Molecular Evolution*, **61**, 531–541.
- Reusch TBH, Häberli MA, Aeschlimann PB, Milinski M (2001) Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature*, **414**, 300–302.
- Reusch TBH, Schaschl H, Wegner KM (2004) Recent duplication and inter-locus gene conversion in major histocompatibility class II genes in a teleost, the three-spined stickleback. *Immunogenetics*, **56**, 427–437.
- Richman AD, Herrera LG, Nash D (2001) MHC class II beta sequence diversity in the deer mouse (*Peromyscus maniculatus*): implications for models of balancing selection. *Molecular Ecology*, **10**, 2765–2773.
- Sato A, Figueroa F, O'hUigin C, Steck N, Klein J (1998) Cloning of major histocompatibility complex (MHC) genes from threespine stickleback, *Gasterosteus aculeatus*. *Molecular Marine Biology and Biotechnology*, **7**, 221–231.
- Sato A, Figueroa F, Murray BW *et al.* (2000) Nonlinkage of major histocompatibility complex class I and class II loci in bony fishes. *Immunogenetics*, **51**, 108–116.

- Scharsack JP, Kalbe M, Harrod C, Rauch G (2007a) Habitat-specific adaptation of immune responses of stickleback (*Gasterosteus aculeatus*) lake and river ecotypes. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 1523–1532.
- Scharsack JP, Kalbe M, Schaschl H (2007b) Characterization of antisera raised against stickleback (*Gasterosteus aculeatus*) MHC class I and class II molecules. *Fish & Shellfish Immunology*, **23**, 991–1002.
- Schaschl H, Wandeler P, Suchentrunk F, Obexer-Ruff G, Goodman SJ (2006) Selection and recombination drive the evolution of MHC class II DRB diversity in ungulates. *Heredity*, **97**, 427–437.
- Schierup MH, Vekemans X, Charlesworth D (2000) The effect of subdivision on variation at multi-allelic loci under balancing selection. *Genetical Research*, **76**, 51–62.
- Schou TW, Permin A, Juul-Madsen HR *et al.* (2007) Gastrointestinal helminths in indigenous and exotic chickens in Vietnam: association of the intensity of infection with the Major Histocompatibility Complex. *Parasitology*, **134**, 561–573.
- Scott RJ (2004) Assortative mating between adjacent populations of threespine stickleback (*Gasterosteus aculeatus*). *Ecology of Freshwater Fish*, **13**, 1–7.
- Setchell JM, Charpentier MJE, Abbott KM, Wickings EJ, Knapp LA (2010) Opposites attract: MHC-associated mate choice in a polygynous primate. *Journal of Evolutionary Biology*, **23**, 136–148.
- Spurgin LG, Richardson DS (2010) How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. *Proceedings of the Royal Society B-Biological Sciences*, **277**, 979–988.
- Takahata N, Nei M (1990) Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. *Genetics*, **124**, 967–978.
- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**, 1596–1599.
- Ward AJW, Webster MM, Hart PJB (2007) Social recognition in wild fish populations. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 1071–1077.
- Webster MM, Goldsmith J, Ward AJW, Hart PJB (2007) Habitat-specific chemical cues influence association preferences and shoal cohesion in fish. *Behavioral Ecology and Sociobiology*, **62**, 273–280.
- Wedekind C, Furi S (1997) Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proceedings of the Royal Society of London Series B-Biological Sciences*, **264**, 1471–1479.
- Wedekind C, Little TJ (2004) The clearance of hidden cestode infection triggered by an independent activation of host defense in a teleost fish. *Journal of Parasitology*, **90**, 1329–1331.
- Wegner KM, Kalbe M, Kurtz J, Reusch TBH, Milinski M (2003a) Parasite selection for immunogenetic optimality. *Science*, **301**, 1343.
- Wegner KM, Reusch TBH, Kalbe M (2003b) Multiple parasites are driving major histocompatibility complex polymorphism in the wild. *Journal of Evolutionary Biology*, **16**, 224–232.
- Wegner KM, Kalbe M, Rauch G, Kurtz J, Schaschl H, Reusch TBH (2006) Genetic variation in MHC class II expression and interactions with MHC sequence polymorphism in three-spined sticklebacks. *Molecular Ecology*, **15**, 1153–1164.
- Wegner KM, Kalbe M, Milinski M, Reusch TBH (2008) Mortality selection during the 2003 European heat wave in three-spined sticklebacks: effects of parasites and MHC genotype. *BMC Evolutionary Biology*, **8**, doi: 10.1186/1471-2148-8-124.
- Westerdahl H (2004) No evidence of an MHC-based female mating preference in great reed warblers. *Molecular Ecology*, **13**, 2465–2470.
- Westerdahl H, Hansson B, Bensch S, Hasselquist D (2004) Between-year variation of MHC allele frequencies in great reed warblers: selection or drift? *Journal of Evolutionary Biology*, **17**, 485–492.
- Woelfing B, Traulsen A, Milinski M, Boehm T (2009) Does intra-individual major histocompatibility complex diversity keep a golden mean? *Philosophical Transactions of the Royal Society B-Biological Sciences*, **364**, 117–128.

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LB was SM's thesis supervisor. His research interests focus on the understanding of patterns and processes of molecular and organismal evolution, and their relevance to conservation. This research was part of SB's honours project (BSc), and SM's PhD thesis on environmental and genetic variance underlying morphological, physiological and behavioural traits in sticklebacks. Sébastien is completing his MSc research on harmful cyanobacteria blooms with CEN. Scott is pursuing his interests in evolutionary ecology as a postdoc with EGRU.

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## Supporting information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Origins of sticklebacks used in mate choice trials.

**Fig. S2** Phylogeny of MHC class IIb sequences for the putative stickleback PBR.

**Fig. S3** Phylogeny of the 276 AA sequences corresponding to the putative PBR of sticklebacks from the St. Lawrence estuary.

**Table S1** PCR conditions used to amplify genomic DNA containing the putative stickleback peptide-binding region (PBR) for MHC Class IIβ loci

**Table S2** Analysis of variance for models describing linear and quadratic relationships between two metrics of individual MHC diversity, the number of unique PBR sequences or the average intra-individual protein (AA) distance and total parasite diversity, defined by the Shannon–Wiener index (H)

**Table S3** Preliminary analyses testing for a potential immune response stimulation effect because of cutting dorsal spine tip and testing for the influences of spine cutting and parasite load on reproductive success

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