

Signals of major histocompatibility complex overdominance in a wild salmonid population

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The major histocompatibility complex (MHC) contains the most variable genes in vertebrates, but despite extensive research, the mechanisms maintaining this polymorphism are still unresolved. One hypothesis is that MHC polymorphism is a result of balancing selection operating by overdominance, but convincing evidence for overdominant selection in natural populations has been lacking. We present strong evidence consistent with MHC-specific overdominance in a free-living population of Arctic charr (*Salvelinus alpinus*) in northernmost Europe. In this population, where just two MHC alleles were observed, MHC heterozygous fish had a lower parasite load, were in better condition (as estimated by a fatness indicator) and had higher survival under stress than either of the homozygotes. Conversely, there was no consistent association between these fitness measures and assumedly neutral microsatellite variability, indicating an MHC-specific effect. Our results provide convincing empirical evidence consistent with the notion that overdominance can be an important evolutionary mechanism contributing to MHC polymorphism in wild animal populations. They also support a recent simulation study indicating that the number of alleles expected to be maintained at an MHC loci can be low, even under strong heterozygote advantage.

Keywords: major histocompatibility complex; overdominance; heterozygote advantage; parasite; condition; survival

1. INTRODUCTION

The extreme polymorphism of the major histocompatibility complex (MHC) genes has fascinated researchers for decades, but the mechanisms of how this extensive genetic variation is maintained are hotly debated (Klein 1986; Hughes & Nei 1988; Potts & Slev 1995; Apanius *et al.* 1997; Edwards & Hedrick 1998; Paterson *et al.* 1998; Wegner *et al.* 2003a). The primary role of the MHC is to encode molecules that recognize foreign proteins, present them to T-cells and initiate an immune response (Lanfear *et al.* 2001; Piertney & Oliver 2006). The crucial function of MHC molecules in the adaptive immune response suggests that pathogens and parasites are the most important agents of selection for MHC polymorphism (Landry & Bernatchez 2001; Wegner *et al.* 2004; Froeschke & Sommer 2005; Harf & Sommer 2005; de Eyto *et al.* 2007; Turner *et al.* 2007).

Hypotheses proposed to maintain MHC polymorphism include the overdominance (heterozygote superiority) hypothesis which predicts that heterozygous individuals

are fitter than individuals homozygous for an MHC allele because two different alleles will identify a broader range of peptides (Doherty & Zinkernagel 1975) compared with one. Although overdominance has been convincingly argued to contribute to the evolution of MHC polymorphism (Doherty & Zinkernagel 1975; Hughes & Nei 1989, 1992; Takahata & Nei 1990), more recent studies suggest that the ability of overdominance to create and maintain MHC polymorphism may be more limited (Hedrick 1999, 2002; Borghans *et al.* 2004; De Boer *et al.* 2004; Stoffels & Spencer 2008). Considerable empirical support has been presented for the importance of MHC variability in pathogen and parasite resistance from studies on humans, or on other vertebrates under experimental conditions (Jeffery & Bangham 2000; Mitton 2002; Bernatchez & Landry 2003; Wegner *et al.* 2003b, 2004; Sommer 2005). However, only a few studies have found experimental evidence for overdominance (Penn 2002; McClelland *et al.* 2003), and unequivocal support for the overdominance hypothesis has been completely lacking in wild animal populations.

In the present study, we investigated the relationship between MHC diversity and fitness in a natural salmonid population. Several studies have reported fitness differences between MHC heterozygotes and homozygotes

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when comparing all different heterozygous or homozygous genotypes in two groups (population heterozygote advantage (Sommer 2005). However, such a group comparison approach does not enable testing of the original allele-specific hypothesis, whereby heterozygotes are expected to be superior to each homozygote class (Doherty & Zinkernagel 1975) and, therefore, cannot be considered as support for overdominance. In fact, population heterozygote advantage can sometimes occur even when the fitness of heterozygotes is lower than that of either corresponding homozygote (Lipsitch *et al.* 2003). In addition, only a handful of studies where a population heterozygote advantage has been observed have combined estimates of MHC diversity with estimates of genome-wide diversity by also assessing assumedly neutral markers (Wegner *et al.* 2003a; Turner *et al.* 2007). Therefore, possible genome-wide effects often cannot be ruled out.

We investigated genetic variability of the MHC class II β peptide-binding region (PBR) in a wild Arctic charr (*Salvelinus alpinus*) population (Lake Peltojärvi, northernmost Finland). Furthermore, we investigated 11 assumedly neutral microsatellite loci and compared the parasite load, condition (fatness) and survival during fishing stress between different MHC and microsatellite genotypes. Our results provide strong empirical evidence consistent with the notion that overdominance can be an important evolutionary mechanism contributing to MHC polymorphism in wild animal populations.

2. MATERIAL AND METHODS

(a) Data collection, measurements and parasitological examination

Arctic charr (*S. alpinus*) individuals were gillnetted in April and September 2006 from Lake Peltojärvi in northern Finland (69°7' N, 26°34' E), which contains a presumably indigenous Arctic charr population (Finnish Game and Fisheries Research Institute, broodstock registry). Gillnets were examined once a day (approximately at the same time every day) and fish were frozen and transported to the laboratory for later analyses. During the gillnetting periods, the total numbers of dead and alive fish were counted. In the laboratory, fish were measured, weighed, sexed, their stomach contents analysed and the adipose fins placed in 95 per cent ethanol for genetic analyses. In addition, *Diphyllbothrium* cysts from the body cavity and muscles were counted and thickness of the belly flap was measured for charr over 300 mm using a micrometer (Mitutoyo Corporation) to 0.01 mm accuracy from four standard locations (two measurements per fillet). Fish less than 300 mm in length ($n = 10$) were excluded from belly flap measurements, as the size of the fillets was too small for the instrument used for thickness to be measured accurately. Fitness measurements and genetic analyses (see below) were conducted blind, i.e. by separate researchers, without one knowing the results of the other.

(b) DNA extraction, major histocompatibility complex primer design and polymerase chain reaction

DNA was extracted from tissue samples as described in Vähä *et al.* (2007). Arctic charr MHC II β sequences were initially characterized using Atlantic salmon designed primers MG7 and ÅL 1002 (Langefors *et al.* 2000). Because cross-species

amplification of Arctic charr MHC with salmon primers proved to be difficult in some cases, Arctic charr sequence-specific primers MHC-int-F and MHC-int-R were designed (electronic supplementary material, table S1). They amplified a 212 bp long fragment of the MHC II β PBR. The primers were designed with the program PRIMER3 (Rozen & Skaletsky 2000), using full-length second exon sequences (see below). The MHC-int-F-primer had a 40 bp 'CG clamp' added to the 3'-end to prevent total denaturation of samples (Sheffield *et al.* 1989) in denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* 1987). The 25 μ l PCR mixture contained approximately 100 ng of DNA, 0.75 U of BioTaq polymerase (Bioline), 12.5 pmol of each primer, 1 \times PCR reaction buffer, 60 pmol of MgCl₂ and 6.25 pmol of dNTPs. The PCR program consisted of an initial 2 min denaturation at 95°C, followed by 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 56°C and extension for 1 min at 72°C. The final extension was for 10 min at 72°C.

(c) Denaturing gradient gel electrophoresis

DGGE was performed using 9.5 μ l of the PCR product in 9 per cent 19:1 acrylamide : bisacrylamide gels containing 1 \times TAE buffer and a gradient of urea and formamide (Hayes *et al.* 1999). The gels were cast with a gravitational gradient maker and the DGGE was performed in a DGGE system buffer tank (C.B.S. Scientific Inc.) filled with 0.5 \times TAE and heated to 60°C. Separation of different alleles was found to be optimal with a 25–55% denaturant gradient and with an electrophoresis running time of approximately 900 Vh. The gels were stained with ethidium bromide for 10 min after the electrophoresis and stained DNA was visualized with a BioRad ChemiDoc XRS imaging system, after which the individuals were genotyped by eye.

(d) Cloning and sequencing

The genotypes of 14 individuals from the Peltojärvi population were confirmed by sequencing directly from both DGGE PCR products and full-length PBR fragments cloned to pGEM-T plasmids (Promega Corporation) according to manufacturers' instructions. The MHC-int-F primer without the CG clamp was used for direct sequencing. The full-length PBRs were amplified with the same PCR program as described above, using salmon-specific primers MG7 and ÅL 1002 (Langefors *et al.* 2000). Up to five clones were sequenced from each individual with an Applied Biosystems 3130xl Genetic Analyzer following the recommendations of the manufacturers, using vector-specific primers M13F and M13R. The sequences were aligned and analysed using Vector NTI Advance 10 (Invitrogen). In all 62 cases, the sequence of the allele(s) matched the genotypes obtained by DGGE. Sequences were deposited in GenBank (accession numbers GQ165716 and GQ165717).

(e) Microsatellite genotyping and data analyses

The level of neutral microsatellite variation in the studied fish was ascertained using 11 microsatellite markers (electronic supplementary material, table S1). The PCR was carried out in two 10 μ l multiplexes using QIAGEN Multiplex PCR Kit according to the manufacturer's instructions with the annealing temperature of the recommended PCR program set to 56°C. The forward primer for each locus was end labelled with a fluorescent dye and a GTTT tail was added to each reverse primer to ensure addition of an A-overhang to each PCR fragment by Taq polymerase (Brownstein *et al.* 1996). Microsatellites were

Table 1. ANCOVA and ANOVA statistics assessing the effect of MHC genotype and season (spring versus autumn) on two fitness measures (1 and 2) and number of sticklebacks in stomach (3), as well as an assessment of associations between survival (dead versus alive) and the two fitness measures (4 and 5).

parameter	analysis	details of the test	<i>n</i>	<i>F</i>	<i>p</i>	
1. number of <i>Diphyllobothrium</i> versus genotype	ANCOVA	genotype ^a	50	4.522	0.017	
		season ^a		0.506	0.481	
		genotype × season		0.662	0.521	
		standard length ^b		40.576	<0.001	
2. belly flap thickness versus genotype	ANCOVA	genotype ^a	37	5.176	0.012	
		season ^a		2.238	0.145	
		genotype × season		0.719	0.495	
		standard length ^b		4.381	0.045	
3. number of sticklebacks in stomach	ANOVA	genotype ^a	50	0.067	0.935	
		season ^a		28.339	<0.001	
		genotype × season		0.509	0.605	
		standard length ^b		6.410	0.016	
4. belly flap thickness versus survival	ANCOVA	survival ^a	37	6.410	0.016	
		standard length ^b		8.413	0.006	
		survival ^a		50	0.825	0.368
		standard length ^b		31.951	<0.001	

^aFixed factor.

^bCovariate.

genotyped using the same methods as described in Vähä *et al.* (2007). The observed and expected heterozygosities were calculated using MICROSATELLITE TOOLKIT (Park 2001), allelic richness and the inbreeding coefficient, F_{IS} , were calculated using FSTAT (Goudet 1995) and the possible deviation from Hardy–Weinberg equilibrium was estimated using GENEPOP (Raymond & Rousset 1995). Severe reductions in the effective size of the populations (i.e. bottlenecks) were tested using the program BOTTLENECK (Piry *et al.* 1999). Statistical significance was tested with three tests: the sign test, the one-way Wilcoxon sign-rank test and the ‘mode-shift’ test. For two-phase mutation model tests, 30 per cent non-stepwise mutations were allowed. A measure of individual inbreeding level, internal relatedness (IR), was calculated according to Amos *et al.* (2001).

(f) Statistical analyses

The effect of MHC genotype and season (fixed factors) on *Diphyllobothrium* parasite numbers, belly flap thickness, number of sticklebacks in stomachs, mean observed microsatellite heterozygosity (H_{OBS}) and IR were studied with ANOVA or ANCOVA, with fish standard length as a covariate (table 1; electronic supplementary material, table S2). The effect of microsatellite genotype (homozygous versus heterozygous) on parasite numbers and belly flap thickness was tested by ANCOVA (electronic supplementary material, table S3). ANCOVA was used also to study the effect of survival status (fixed factor) on belly flap thickness, *Diphyllobothrium* numbers (table 1) and H_{OBS} . As the seasonal differences in belly flap thickness and *Diphyllobothrium* numbers were not significant, the spring and autumn samples were combined in ANCOVAs studying the effect of survival status. It was verified that none of the interactions between the covariate and any of the factors was significant (equal slopes assumption). Normality was assessed using Shapiro–Wilk statistics and when needed, natural-logarithm transformation was applied to the dependent variable to satisfy the requirement of normal distribution. Tukey’s least significant difference post hoc tests were used to study between-group differences in ANCOVAs. Associations between *Diphyllobothrium* numbers and belly flap thickness and between H_{OBS} and *Diphyllobothrium* abundance or

belly flap thickness were studied with partial correlations, fish standard length being a controlling variable. All presented *p*-values are from two-tailed tests with $\alpha = 0.05$.

To test if the proportion of survived fish among MHC heterozygotes differed from both MHC homozygotes, logistic regression with simple contrast was used with the forward stepwise method based on the log-likelihood ratio so that the spring and autumn samples were combined, and the genotype and fish standard length were used as explanatory variables (electronic supplementary material, table S4). Possible survival differences between microsatellite heterozygotes and homozygotes in seven microsatellite loci were investigated in a similar manner (each of the seven polymorphic loci analysed separately), but without contrasting (electronic supplementary material, table S5).

3. RESULTS

(a) Genetic variation of Arctic charr in study lakes

The level of genetic variability in Lake Peltojärvi was low: only two MHC II β alleles (*Saal-DAB*001* and *Saal-DAB*002*) were identified in the 50 individuals assessed and the mean number of alleles in 11 microsatellite loci known to be polymorphic in some north European Arctic charr populations was 2.6 (table 2; electronic supplementary material, table S6). The sequence divergence between the two MHC PBR alleles was 29 nucleotides (9%) and 16 amino acids (13%) with a dN/dS ratio of 3.17 (electronic supplementary material, figure S1). To assess the uniqueness of the low MHC variability found in Lake Peltojärvi, we also genotyped Arctic charr from three Norwegian lakes (26–35 individuals per population) and also observed relatively low levels of variation; 3–9 MHC alleles and an average of 2.5–6.2 microsatellite alleles per locus (table 2; electronic supplementary material, table S6). There was no evidence for recent population bottlenecks in Lake Peltojärvi (all tests $p > 0.24$). In addition, there were no indications of inbreeding (as estimated by F_{IS} , electronic supplementary material, table S6) in any population. Also neither the

Table 2. Diversity indices for the MHC locus and microsatellites (averaged over 11 loci) in Arctic charr populations of Lake Peltojärvi and three presumably unstocked small Norwegian lakes from similar latitudes (Primmer *et al.* 1999). See electronic supplementary material, table S6 for more detailed information. no. A, number of observed alleles; Ar, allelic richness; H_o , observed heterozygosity.

	Peltojärvi 69°07' N, 26°34' E			Haukejavri 69°57' N, 29°14' E			Lisma 70°08' N, 28°04' E			Bluevatnet 70°37' N, 30°05' E			mean			
	no. A	Ar	H_o	no. A	Ar	H_o	no. A	Ar	H_o	no. A	Ar	H_o	total no. A	A	Ar	H_o
MHC II β	2	2.0	0.38	3	3.0	0.38	6	5.9	0.56	9	9.0	0.52	16	5	5.0	0.46
microsatellites	2.6	2.5	0.25	2.5	2.8	0.23	3.5	3.4	0.36	6.2	6.4	0.53	8.6	3.92	6.0	0.34

mean observed microsatellite heterozygosity (H_{OBS}) nor IR differed between the MHC genotypes in Lake Peltojärvi charr (ANCOVA, $F_{1,49} = 0.070$, $p = 0.932$ and $F_{1,49} = 0.031$, $p = 0.969$, respectively, electronic supplementary material, table S2).

(b) *Diphyllbothrium* abundance

There were up to 1500 *Diphyllbothrium* (Cestoda) plerocercoid cysts, mainly 4–8 mm in diameter each, encapsulated by the host in the body cavity and muscles of the charr. We opened and examined approximately five *Diphyllbothrium* cysts from most of the fish finding plerocercoids resembling *Diphyllbothrium dendriticum*, but we cannot exclude the possibility that *Diphyllbothrium ditremum* also occurred in the charr. *Diphyllbothrium* plerocercoids were clearly the most abundant parasite in the charr population. Three other parasite taxa were also detected—*Eubothrium salvelini* (Cestoda) in the intestine, *Diplostomum* sp. (Trematoda) in the eyes and *Acanthobdella peledina* (Hirudinae) on the skin—but the materials and numbers of infected individuals were insufficient for statistical analyses for those parasites. We found a significant difference in the mean number of *Diphyllbothrium* cysts between the three MHC genotypes (ANCOVA, $F_{1,49} = 4.522$, $p = 0.017$, table 1). Post hoc tests indicated that parasite load was lower in MHC heterozygotes than in either of the homozygotes ($p = 0.007$ compared with *Saal-DAB*001* and $p = 0.043$ for *Saal-DAB*002*), but there was no difference between the homozygotes ($p = 0.439$) (figure 1a). The effect of sampling period (April versus September) was not significant, but parasite load increased with the covariate, fish standard length (table 1). However, there was no difference in the mean length of individuals between different MHC genotypes (electronic supplementary material, table S2 and supplementary data).

The number of digested three-spined sticklebacks (*Gasterosteus aculeatus*) in the stomachs of Arctic charr did not differ between MHC genotypes (ANOVA, $F_{1,49} = 0.067$, $p = 0.935$) (table 1). Sticklebacks are the most abundant prey and presumably the main vector of *Diphyllbothrium* in those large size classes included in the present study (210–595 mm) (electronic supplementary material, supplementary data). These observations suggest that the lower *Diphyllbothrium* abundance in MHC heterozygotes is due to higher parasite resistance rather than lower exposure among the heterozygous fish.

(c) Thickness of the belly flap and survival

Thickness of the belly flap differed between the MHC genotypes (ANCOVA, $F_{1,36} = 5.176$, $p = 0.012$,

table 1), with heterozygotes having a thicker belly flap than both *Saal-DAB*001* ($p = 0.011$) and *Saal-DAB*002* homozygotes ($p = 0.012$), but the belly flap thickness of homozygotes did not differ between each other ($p = 0.940$) (figure 1b). In the data combined over spring and autumn samples, to test if survival during gillnetting was higher in MHC heterozygotes than in either of the homozygotes the final logistic regression model included the variable 'genotype' but not 'fish standard length' (forward stepwise method based on likelihood ratio, $-2 \log$ likelihood = 49.318, Nagelkerke $R^2 = 0.260$). Thus, the result of logistic regression analysis indicated that survival of fish during gillnetting stress was significantly higher among MHC heterozygotes (95%, $n = 19$) than in either of the homozygotes (65%, $n = 17$, and 50%, $n = 14$, for *Saal-DAB*001* and *Saal-DAB*002*, respectively) (figure 1c; electronic supplementary material, table S4). Overall, these findings indicate higher fitness of MHC heterozygote individuals (see also Penn *et al.* 2002).

(d) Associations between fitness measures

There was a statistically significant negative correlation between the number of *Diphyllbothrium* and the thickness of the belly flap (partial correlation, with the effect of fish standard length partialled out, $r = -0.430$, $n = 37$, $p = 0.009$). In addition, the mean (\pm s.e.) thickness of the belly flap was lower among charr that died during the gillnetting (2.82 ± 0.15 mm) than in those that survived (3.50 ± 0.20 mm) (ANCOVA, $F_{1,36} = 6.410$, $p = 0.016$, table 1, figure 2). Although the dead and alive fish did not differ significantly in their numbers of *Diphyllbothrium* cysts (ANCOVA, $F_{1,49} = 0.825$, $p = 0.368$), the trend was in the expected direction (mean \pm s.e.; 201 ± 103 and 90 ± 24 , respectively).

(e) Genome-wide effects

There was no dependence between locus-specific heterozygosity at any of the seven polymorphic microsatellites and belly flap thickness (ANCOVAs, $p > 0.050$, in all cases, electronic supplementary material, table S3) or survival during gillnetting (logistic regression analyses, electronic supplementary material, table S5). Furthermore, there was no dependence between H_{OBS} and *Diphyllbothrium* abundance or belly flap thickness (partial correlations, fish standard length partialled out, $r = -0.063$, -0.080 , $n = 49$ and 37 and $p = 0.671$ and 0.641 , respectively) or between H_{OBS} and survival (ANCOVA, $F_{1,49} = 1.406$, $p = 0.242$). Abundance of *Diphyllbothrium* cysts was significantly associated with

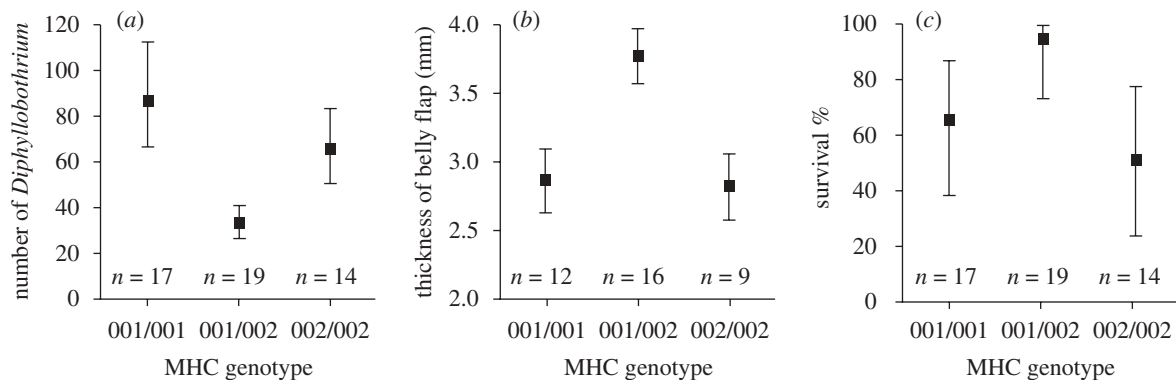


Figure 1. (a) Size-adjusted mean (\pm s.e.) number of *Diphyllobothrium* plerocercoid cysts, (b) mean (\pm s.e.) belly flap thickness and (c) survival during gillnetting (proportion of survived individuals \pm 95 per cent CI) in Arctic charr with different MHC genotypes.

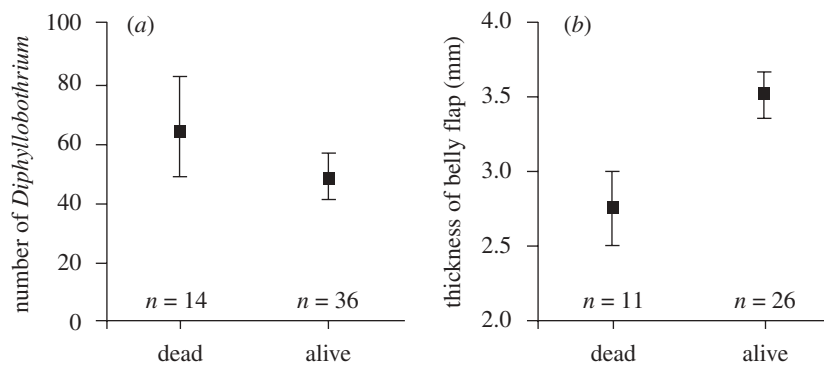


Figure 2. (a) Size-adjusted mean (\pm s.e.) number of *Diphyllobothrium* plerocercoid cysts and (b) mean (\pm s.e.) belly flap thickness in dead and survived Arctic charr.

just one microsatellite locus (ANCOVA, $F_{1,48} = 5.687$, $p = 0.007$, electronic supplementary material, table S3). However, post hoc tests revealed that the parasite number of heterozygotes was only lower than the average of the homozygotes, but not lower than both ($p = 0.002$ and 0.092), i.e. overdominance was not detected. In addition, this rate of significance at assumedly neutral microsatellite loci (one significant association out of 21 tests conducted) is in line with the rate expected owing to the type I error, a conclusion which is also supported by the fact that this locus was associated with only one of the three fitness measures. Thus, our results indicate an MHC-specific effect, rather than a genome-wide heterozygote advantage.

4. DISCUSSION

Our results indicate clear signals of MHC-specific overdominance with respect to three different parameters: MHC heterozygous charr had significantly lower *Diphyllobothrium* plerocercoid numbers, thicker belly flaps and higher survival during gillnetting than either of the homozygotes, which together indicate higher fitness of MHC heterozygote individuals. According to the overdominance theory, heterozygotes are expected to have a selective advantage compared with homozygotes, because two different alleles will identify a broader

range of pathogens and parasites than one (Doherty & Zinkernagel 1975).

Energy reserves often play an important role in determining fitness. Lipid stores are the primary energy reserves of fish preparing to reproduce (Rowe & Thorpe 1990) and the size of these stores may determine the survival probability during unfavourable feeding conditions, which are not uncommon in Arctic conditions. In salmonids, the major and most readily mobilized fat reserve is in the belly flap (Nanton *et al.* 2007). Thus, the size of this lipid store can be regarded as an indicator of the overall condition of the fish. The negative associations between the measured fitness parameters (§3) suggest that the impact of *Diphyllobothrium* parasites on individual fitness may be high. This is not surprising given (i) the high observed numbers of these relatively large parasites in the charr population; (ii) the fact that they are located in the muscles and body cavity, and migrate to their final site of infection through the tissues of the host; and (iii) they have been shown to cause severe pathological tissue damage and mortality in salmonid fishes (Rahkonen *et al.* 1996).

We did not find any evidence that our results could be somehow related to inbreeding or population bottlenecks in the Lake Peltojärvi charr population, although the genetic variability in both MHC and microsatellite loci was low. Low levels of variation are not unusual for northern Arctic charr populations (table 2; electronic

supplementary material, table S6) as has also been demonstrated for MHC in a North American Arctic charr population (Conejeros *et al.* 2008). Arctic charr juveniles are able to discriminate siblings and non-siblings with respect to both MHC II-linked odours and odours of some other genes when fish cannot rely on information from MHC class II alleles (Olsén *et al.* 2002; see also Amos & Balmford 2001; Hoffman *et al.* 2007). This suggests that Arctic charr are capable of avoiding mating with close relatives, which could reduce the probability of inbreeding also in Lake Peltojärvi. Although heterozygote deficiencies were observed at the MHC locus in some of the other Nordic charr populations, this is likely to be due to technical difficulties in visualizing all heterozygotes using the DGGE method (Conejeros *et al.* 2008), as the DGGE conditions were optimized primarily for the Lake Peltojärvi population and estimates for other lakes are provided primarily to indicate levels of genetic diversity.

A closer examination of genotype frequencies in the spring and autumn samples provides preliminary evidence that the selective regime in Lake Peltojärvi may be more complex than simple parasite-driven overdominance, as has been suggested in other studies (Froeschke & Sommer 2005). More specifically, there was in fact a strong trend towards a decrease in heterozygotes and an increase in *Saal-DAB*001* homozygotes in the autumn sample, even though the mortality of homozygotes was substantial (figure 1c). If the mortality differences between homozygotes and heterozygotes are used to estimate a selection coefficient to predict expected genotype frequencies in the autumn (second sampling), this trend is in fact significantly different from the expected genotype frequencies (data not shown).

Although a longer time series is required before any firm conclusions can be drawn, possible explanations include: seasonal variation in selection that would result in selective mortality during one time period being a poor predictor of selection coefficients during other time periods. Second, other non-detected parasites against which *Saal-DAB*001* homozygous fish were most resistant could explain an increase in *Saal-DAB*001* homozygotes between spring and autumn. Third, the observed mortality rates during gillnetting may not accurately describe the actual selective advantage of MHC heterozygotes. In this case, the higher mortality of homozygotes may simply reflect their lower survival probability (e.g. higher oxygen demand of highly parasitized fish, see e.g. Booth *et al.* 1993) when caught in gillnets, but not necessarily their higher mortality in more natural conditions. Fourth, if MHC heterozygotes, i.e. fish with lower parasite loads, were better able to avoid capture in gillnets or have better ability to escape from the nets, then the gillnet sample would underestimate the true frequency of heterozygotes. Finally, it is also feasible that the genotype deviations may be simply due to the pooling of samples from different time periods and/or locations, i.e. a possible Wahlund effect. This is further supported by the observation that allele frequencies and also heterozygosity levels at a number of microsatellite loci differed by a similar amount or even more between seasons than did MHC frequencies (data available from CRP on request). Continued monitoring of the population over a longer period to create an extended time series may be of use to distinguish between these possible explanations in the future.

Overdominance is usually assumed to emerge only after simultaneous coinfections when many allele-specific susceptibilities to pathogens will be masked by the resistant allele in heterozygotes (McClelland *et al.* 2003). Thus, in dual infections different alleles should have opposite susceptibility profiles (the dual-infection heterozygote superiority, DIHS hypothesis). The present results indicate overdominance with respect to a single parasite taxon, *Diphyllbothrium*, which could be due to the considerable genetic variation observed within local populations of *Diphyllbothrium* (deVos *et al.* 1990). In helminths, sexual reproduction creates and maintains high antigenic diversity and may rapidly generate strain diversity, which permits evasion of host immunity (Galvani 2003; Galvani *et al.* 2001, 2003). Thus, different parasite genotypes may have different surface antigens, giving possibly a selective advantage to MHC heterozygotes in recognizing a broader range of intra-specific parasite genotypes. Therefore, even if there was only one *Diphyllbothrium* species in Lake Peltojärvi charr, our finding does not necessarily contradict the DIHS hypothesis because it is possible that different MHC alleles may have different levels of recognition degeneracy (i.e. overlap) for a particular pathogen, or pathogen strains within the same species, and hence, overdominance is feasible based on the effects of a single species (Neff *et al.* 2008; Stoffels & Spencer 2008). The higher variation in homozygotes, compared with heterozygotes, for parasite load, belly flap thickness and survival under stress (figure 1) is a further indication that there may be allele-specific variation in fitness (i.e. susceptibility profiles) against different parasite genotypes, although the overall fitness of homozygote genotypes would remain the same (Stoffels & Spencer 2008).

We have demonstrated signals clearly consistent with MHC-specific overdominance in a free-living animal population. Interestingly, Oliver *et al.* (2009) recently demonstrated that in a water vole (*Arvicola terrestris*) population with a low level of MHC variation, MHC heterozygote individuals were infected by fewer parasite types than homozygotes. Therefore, this is the second study within a short period of time presenting empirical evidence supporting the overdominance hypothesis in a population with low MHC polymorphism. But why should such a signal be observed in this population when numerous other studies have failed to detect such an association? A recent simulation study (Stoffels & Spencer 2008) provides a possible answer to this question. Namely, by characterizing the function of MHC molecules by the sets of parasites they recognize (recognition sets) as well as considering the effects of effective population size and genetic drift, Stoffels & Spencer were able to demonstrate that for populations with $N_e = 1000$ (the smallest N_e considered), the number of alleles expected to be maintained at an MHC locus can be less than five, even with a relatively strong heterozygote advantage.

The lower level of MHC diversity observed in this northernmost Arctic charr population, which appears to be a feature of salmonid populations in northern regions (Dionne *et al.* 2007), falls within the range of parameters suggested by Stoffels & Spencer (2008) where a strong effect of overdominance can be expected. Similarly, Lewontin *et al.* (1978) demonstrated that the proportion of stable heterosis equilibria is highest when the number

of alleles in a population is just two. In addition, the lower levels of variability may simplify analyses and contribute to the ability to observe such an allele-specific association with moderate sample sizes (Apanius *et al.* 1997; Oliver *et al.* 2009). Thus, our results indicate that overdominance can be an important evolutionary mechanism contributing to MHC diversity in wild populations. However, as a two-allele system only enables the investigation of one class of heterozygotes, future analyses, for example, including the investigation of additional populations in the region would be useful to determine the generality of our findings and for further investigating how MHC–pathogen interactions may affect the occurrence of overdominance.

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