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MHC-mediated mate choice increases parasite resistance in salmon

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Natural (parasite-driven) and sexual selection are thought to maintain high polymorphism in the genes of the major histocompatibility complex (MHC), but support for a link between mate choice, MHC variation and increased parasite resistance is circumstantial. We compared MHC diversity and *Anisakis* loads among anadromous Atlantic salmon (*Salmo salar* L.) returning to four rivers to spawn, which had originated from natural spawning (parents allowed to mate freely) or artificial crosses (parents deprived from the potential benefits of mate choice). We found that the offspring of artificially bred salmon had higher parasite loads and were almost four times more likely to be infected than free-mating salmon, despite having similar levels of MHC diversity. Moreover, the offspring of wild salmon were more MHC dissimilar than the offspring of artificially crossed salmon, and uninfected fish were more dissimilar for MHC than infected fish. Thus, our results suggest a link between disassortative mating and offspring benefits and indicate that MHC-mediated mate choice and natural (parasite-driven) selection act in combination to maintain MHC diversity, and hence fitness. Therefore, artificial breeding programmes that negate the potential genetic benefits of mate choice may result in inherently inferior offspring, regardless of population size, rearing conditions or genetic diversity.

Keywords: major histocompatibility complex; mate choice; good genes; compatible genes; parasite resistance; salmon

1. INTRODUCTION

The importance of indirect genetic benefits (potential benefits for the offspring) in the evolution of mate choice is a contentious issue (Charmantier & Sheldon 2006). While a number of studies have long suggested a positive relationship between mate choice and offspring fitness (Reynolds & Gross 1992), distinguishing between indirect genetic benefits and other drivers of sexual selection has proved problematic (Kokko 2001). For example, females may choose to mate with the most attractive males to accrue 'good genes' for their offspring (Iwasa *et al.* 1991), but also to enhance offspring attractiveness (Fisherian runaway selection; Lande 1981). Assessing indirect fitness benefits of mate choice should involve measuring not only parent but also offspring fitness, which is often neglected (Kokko 2001).

The genes of the major histocompatibility complex (MHC) represent a good model to test the indirect genetic benefits of mate choice because they are extremely polymorphic and play a crucial role in the initiation of the immune response in vertebrates (Klein 1986). Since MHC genes are also linked with the generation of individual odours (Wedekind & Furi 1997), they could provide clues for choosing dissimilar mates in order to increase offspring genetic diversity, avoid genetic incompatibility (Tregenza & Wedell 2000) or maximize immunocompetence (Penn & Potts 1999; Penn 2002).

Natural and sexual selection are thought to maintain MHC polymorphism in vertebrates, but their relative roles are open to debate. Natural (parasite-driven) selection could promote MHC diversity through heterozygote advantage, rare-allele advantage or allele optimality (Hedrick & Kim 2000). According to the heterozygote advantage hypothesis, heterozygotes could benefit by being able to respond to a greater variety of pathogens (Penn *et al.* 2002). However, having too many alleles may increase the risk of an autoimmune response, and this may favour an optimum intermediate level of MHC diversity (Penn & Potts 1999). Rare alleles could have an advantage in a frequency-dependent scenario if pathogens target the most common alleles (de Campos-Lima *et al.* 1993).

On the other hand, sexual selection could also drive MHC diversity through mate choice and indirect genetic benefits if mates are trying to avoid kin, maximize genetic compatibility or enhance immunocompetence of their offspring. Several studies have shown evidence of MHC-related mate choice in many vertebrates (Potts *et al.* 1991; Reusch *et al.* 2001; Roberts & Gosling 2003), but its adaptive significance is not clear. Analysis of point mutations (e.g. Consuegra *et al.* 2005) and associations between specific MHC alleles and pathogen resistance (e.g. Wedekind *et al.* 2004; Sommer 2005) provide strong support for the role of natural selection in maintaining MHC diversity, but indirect genetic benefits assume the existence of good genes or 'compatible genes', evidence of which is more controversial (Neff & Pitcher 2005). Sexual and natural selection may act in concert (Wegner *et al.* 2004) if, for example, females benefit from choosing males that increase MHC offspring diversity and hence resistance to parasites. Indeed, females appear to choose mates on the basis of MHC dissimilarity (Penn & Potts 1999), or the number of alleles that they carry (Reusch *et al.* 2001).

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The molecular complexity associated with MHC studies, particularly in mammals with several linked class I and class II genes, makes it difficult to disentangle the precise evolutionary mechanisms of these genes and there is a need for studies that relate the roles of sexual and parasite-driven natural selection on the MHC (Penn & Potts 1998). Studies on three-spined sticklebacks (*Gasterosteus aculeatus*) provide some of the best non-human evidence of the potential combined action of natural and sexual selection on MHC genes. Female sticklebacks seem to choose males that result in offspring with intermediate MHC variability (Reusch *et al.* 2001), perhaps because intermediate MHC diversity tends to be associated with lower parasite loads (Wegner *et al.* 2003). Yet, evidence for indirect genetic benefits of MHC-related mate choice is limited, and we know of no study directly linking parental mate choice with offspring MHC diversity and offspring parasite resistance.

Here, we test whether MHC-mediated mate choice may confer increased parasite resistance to offspring, as predicted by the indirect genetic benefits hypothesis. We used Atlantic salmon (*Salmo salar*, L.) as a model organism, as previous studies had shown evidence of MHC disassortative mating (Landry *et al.* 2001), as well as an association between specific MHC alleles and pathogen resistance (Langefors *et al.* 2001; Lohm *et al.* 2002; Grimholt *et al.* 2003). Atlantic salmon is also particularly suited for MHC studies as it represents the minimal essential MHC in fishes, with only two unlinked MHC class I and class II genes expressed (Shum *et al.* 2001). To test for indirect genetic benefits, we compared the abundance of a marine nematode (*Anisakis* spp.) in the offspring of free mating (wild) and artificially spawned (hatchery) salmon returning to rivers to spawn. *Anisakis* loads show substantial variation between hosts (MacKenzie 2002) and since the parasite does not reproduce in fish (paratenic host), parasite counts are thought to represent the balance between infection and clearing rates.

In the absence of mate choice, we expected to find increased parasite susceptibility among the offspring of artificially spawned fish compared with naturally spawning conspecifics. Furthermore, we reasoned that if parasite-driven natural selection was involved in mate choice, MHC dissimilarity would be positively associated with parasite resistance and salmon allowed to mate freely would show more MHC dissimilarity than those prevented from choosing mates.

2. MATERIAL AND METHODS

(a) *Sample collection and parasite screening*

We collected 245 anadromous Atlantic salmon spawners (122 males, 123 females; 55–97 cm in fork length) returning to four neighbouring rivers in northern Spain (Ason, $n=94$; Pas, $n=59$; Nansa, $n=79$; Deva, $n=22$) during the period 1997–2001 (annual sample size=1–19), as described in Consuegra & Garcia de Leaniz (2007). Salmon spawners included wild fish derived from natural spawning ($n=167$), as well as hatchery-reared fish ($n=78$) from the same cohorts obtained by separately crossing 10–60 females with 2–5 different males from each river each year. Artificially bred fish (termed 'hatchery') were stocked as 8- to 10-month-old juveniles (0+) in their parent rivers and were clearly distinguished from naturally bred fish (termed 'wild') by the absence of an adipose fin. Hatchery and wild fish did not differ in size (t-test$=-0.170$, d.f.$=243$, $p=0.865$), but there was a higher proportion of males among hatchery (64%) than among wild fish (43%; Fisher's exact probability test, $p=0.003$). After stripping, we killed them by a blow on the head and examined them for the presence of internal ascarid parasites in the body cavity and internal organs, but excluding the musculature (Beverley-Burton & Pippy 1978). Ascarid counts were made on a subsample of 80 fish (45 wild and 35 hatchery) and a sample of 37 ascarids was preserved in ethanol for species identification (Guzmán Díez, Fundación AZTI, 48395 Sukarrieta, Spain).

We examined three measures of parasite infection in salmon (Margolis *et al.* 1982): parasite prevalence (percentage of salmon infected); parasite intensity (average number of parasites per infected salmon); and parasite load (average number of parasites per salmon).

(b) *MHC analysis*

We examined variation at the exon 2 (α-1 domain) of the MH class II-α (*Sasa*-DAA) gene (containing the peptide-binding sites). Genomic DNA was isolated from fin and muscle samples using the Wizard SV96 Genomic Purification kit (Promega). We amplified a 214 bp region spanning most of the exon 2 as in Consuegra *et al.* (2005). PCR products were run in a 1% agarose gel and DNA from bands of the expected size was purified using the Qiaquick Gel Purification kit (Qiagen). Both strands of the purified products were sequenced with the same amplification primers using the BigDye Terminator Sequencing kit v. 1.0. Sequences were run at the DNA Sequencing Facility at Oxford University. Nucleotide sequences were aligned using BioEdit v. 5.0.9 and phylogenetic analyses were conducted with MEGA v. 3.1 (Kumar *et al.* 2004).

(c) *Statistical treatment*

Parasite counts and fork length were log transformed (log+0.5) prior to the analysis to improve normality (Rauch *et al.* 2006). We combined annual samples from the four neighbouring rivers to increase statistical power, as there were no significant differences in parasite counts between populations in either wild ($p=0.641$) or hatchery ($p=0.488$) fish. We employed logistic regression and analysis of odds ratio to examine the effects of mate choice (free mate versus no choice), sex and body size on probability of infection and the Fisher's exact probability test to compare differences in the proportion of infected fish between wild and hatchery salmon. The effect of mate choice on parasite loads was examine through two-way ANOVA, with fork length as covariate and sex and fish origin (hatchery and wild) as factors. Associations between specific MHC alleles and infection status (infected versus not infected) were tested by the log-likelihood ratio test (G-test).

The program DnaSP (Rozas *et al.* 2003) was used to estimate non-synonymous substitution rates (K_a) between DAA alleles within individuals as a measure of individual MHC dissimilarity. We then estimated the probability of random MHC allelic dissimilarity (K_a) in hatchery and wild fish by allele permutations in 100 random populations simulated using GENETIX, and compared this against the observed K_a values in each population. Differences in mean K_a between hatchery and wild fish were tested for statistical significance with a bootstrapping t-test implemented in Rundom Pro (Jadwiszczak 2007) and one-way ANOVA

implemented in SPSS v. 13. In order to overcome the problems posed by unequal variances and small number of non-infected hatchery fish, we compared K_a between infected and non-infected fish using two separate variance bootstrapping *t*-tests using: (i) the residuals of a one-way ANOVA with origin as a fixed factor (to account for differences in the proportion of infected fish of wild and hatchery origin) and (ii) only wild fish (subjected to mate choice).

3. RESULTS

(a) *Parasite prevalence*

Ascarids found in returning Atlantic salmon had a mean length of 26.6 mm (s.d. = 3.11) and a mean width of 0.63 mm (s.d. = 0.09), and based on head morphology and other external features, they were classified as third larval stages of *Anisakis simplex sensu stricto* (Guzmán Díez 2002, personal communication, Fundación AZTI, 48395 Sukarrieta, Spain). However, given the taxonomic uncertainty of this parasite complex (D'Amelio *et al.* 2000), we refer to them as *Anisakis* sp.

We found only one case (1/75 = 1.3%) of a parasitized fish without *Anisakis* in the digestive system and are therefore confident that screening of the digestive system and internal organs can be used to accurately determine the infection status of salmon. *Anisakis* prevalence was high in both groups but differed significantly (Fisher's exact probability test, $p = 0.015$) between artificially bred (96%) and naturally spawning (86%) fish. Logistic regression indicated that the probability of infection was significantly lower in wild than in hatchery fish ($p = 0.030$), when statistically controlling for differences in sex ratio ($p = 0.493$) and body size ($p = 0.462$). Analysis of odds ratio indicated that artificially bred salmon were on average 3.95 times more likely to be infected with *Anisakis* than free mating salmon (95% CI = 1.14–13.70), suggesting that mate choice influenced risk of infection.

(b) *Parasite loads*

The number of *Anisakis* per salmon ranged from 0 to 38 and was significantly lower (figure 1; $p = 0.001$) among wild (mean = 6.62, s.d. = 1.36) than hatchery fish (mean = 12.14, s.d. = 1.48), when statistically controlling for differences in body size ($p = 0.326$). Neither the sex ($p = 0.863$) nor the interaction between sex and origin ($p = 0.179$) had any significant effect on parasite loads. Parasite intensity (i.e. excluding uninfected fish) was also significantly lower among the offspring of salmon that were allowed to mate freely than among salmon deprived of mate choice ($p = 0.034$), when controlling for differences in body size ($p = 0.805$). As with parasite load, neither the sex ($p = 0.352$) nor the interaction between sex and origin ($p = 0.147$) had a significant effect on parasite intensity.

(c) *Effect of MHC diversity*

We sequenced 86 individuals (51 adults of wild origin and 35 adults derived from hatchery crosses) and found high levels of variability at the *Sasa*-DAA gene. We observed 16 different alleles, 6 of which had not been described previously in Atlantic salmon and have been labelled *Sasa*-DAA*1301 to *Sasa*-DAA*1801 (GenBank accession nos., EU043353–EU043358). Overall heterozygosity was high ($H_e = 0.860 \pm 0.065$) and did not differ significantly ($p > 0.05$) between hatchery ($H_e = 0.866 \pm 0.095$) and wild ($H_e = 0.846 \pm 0.087$) fish. Numbers of alleles were the same in both groups ($N_a = 14$), and allelic richness was slightly higher in hatchery ($R_a = 14.0$) than in wild ($R_a = 12.8$) fish. Thus, the offspring of hatchery and wild fish had high and similar levels of genetic diversity with respect to the MHC class II gene. On the other hand, *Anisakis* loads were not statistically different between heterozygous and homozygous individuals (*t*-test $F_{1,78} = 0.605$, $p = 0.439$) and did not differ between specific MHC alleles ($F_{6,123} = 1.541$, $p = 0.170$), but differed only between artificially crossed and free mating salmon ($F_{1,123} = 8.374$, $p = 0.005$; figure 2). We also failed to find a significant association between specific MHC alleles and resistance to infection (figure 3*a*, wild fish: $G = 1.855$, d.f. = 3, $p = 0.603$; figure 3*b*, hatchery fish: $G = 3.225$, d.f. = 2, $p = 0.199$).

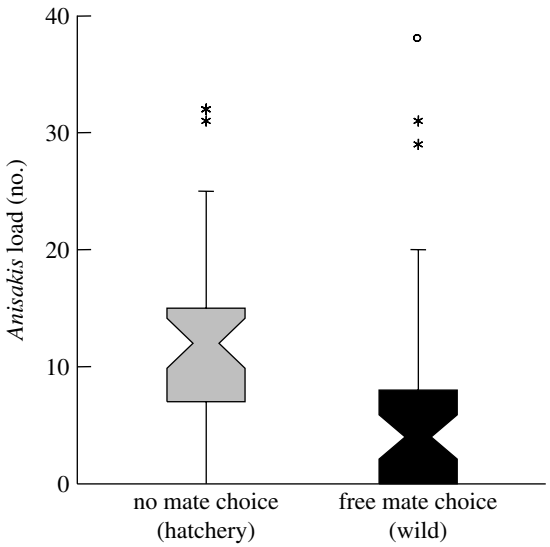

Figure 1. Variation in abundance of *Anisakis* per salmon (median parasite load) in the progeny of wild and hatchery Atlantic salmon returning to rivers to spawn. Box and whisker plots show median values with notches extending to 95% CI around the median, first and third quartiles (boxes), 90% of values (whiskers) and extreme data points (asterisks and circles). Compared with artificially bred salmon deprived of mate choice, the offspring of wild salmon that were allowed to mate freely show significantly lower parasite loads ($p < 0.001$).

(d) *Effect of MHC dissimilarity*

Analysis of the average rate of non-synonymous substitutions (K_a) indicated that MHC allelic dissimilarity was significantly higher than would be expected by chance among the progeny of wild fish ($K_a = 0.052$, $n = 51$, s.e. = 0.003, $p < 0.001$; figure 4*a*), but not that of hatchery fish, which conformed to random expectations ($K_a = 0.041$, $n = 35$, s.e. = 0.004; $p = 0.420$; figure 4*b*). Thus, despite having similar levels of genetic diversity, the progeny of wild fish were more MHC dissimilar than that of artificially crossed salmon (bootstrap *t*-test = 2.196, $p = 0.031$; 10 000 randomizations). Moreover, among wild fish, non-infected salmon were more MHC dissimilar ($K_a = 0.054$, s.e. = 0.002, $n = 17$) than infected individuals ($K_a = 0.048$, s.e. = 0.004; $n = 34$; bootstrap *t*-test = 2.46; $p = 0.022$, 10 000 randomizations). Differences in MHC dissimilarity between infected and uninfected fish (figure 5) were also significant when the effect of stock

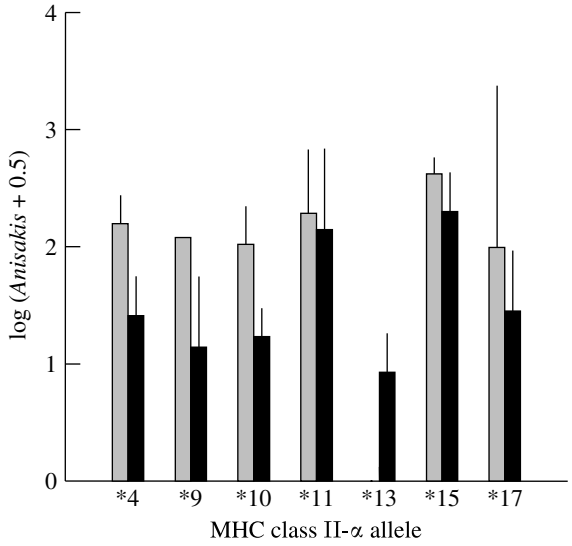

Figure 2. Mean *Anisakis* loads (s.e.) associated with different MHC alleles in the offspring of artificially reared (hatchery, grey bars) and free mating (wild, black bars) salmon. Alleles with low prevalence (less than or equal to three individuals) were omitted from analysis. Parasite loads do not differ between specific alleles ($p=0.170$), only differ between the two types of fish ($p=0.005$).

origin (wild versus hatchery) was statistically controlled by the analysis of K_a residuals (bootstrap t-test$=2.59$; $p=0.012$, 10 000 randomizations).

4. DISCUSSION

To our knowledge, our work represents the first study carried in a natural fish population suggesting a causal link between mate choice, MHC variation and offspring fitness. We addressed this by manipulating mate choice and comparing MHC variability and parasite susceptibility between the progeny of wild salmon (able to mate freely) and hatchery-bred fish (deprived of the potential benefits of mate choice). The parasite we targeted (*Anisakis* sp.) is a marine nematode commonly found in salmon (Bakke & Harris 1998), which act as paratenic intermediate host. Screening of a marine (rather than of a freshwater) parasite minimized potential confounding effects due to possible differences in early rearing between hatchery and wild fish. Fish react to *Anisakis* by producing apoptosis-inducing proteins in infected tissues (Jung *et al.* 2000), suggesting that *Anisakis* can seriously hamper fish health.

Salmonids do not show parental care, and since males provide only genes for the offspring, indirect genetic benefits may be particularly important in this family. Yet, despite numerous studies on MHC-mediated mate choice (reviewed in Jordan & Bruford 1998; Penn 2002), there is little evidence for MHC indirect genetic benefits. Females could benefit from choosing MHC-dissimilar males by increasing MHC diversity of their offspring—and thereby improving offspring immunocompetence (Apanius *et al.* 1997)—or by decreasing the risks of inbreeding or maximizing genetic compatibility (Tregenza & Wedell 2000). Strong homing behaviour (i.e. the ability to return to reproduce to the place of birth) should favour the recognition and avoidance of close mates, but a promiscuous mating system argues against a major role for inbreeding avoidance in the evolution of salmonid life histories

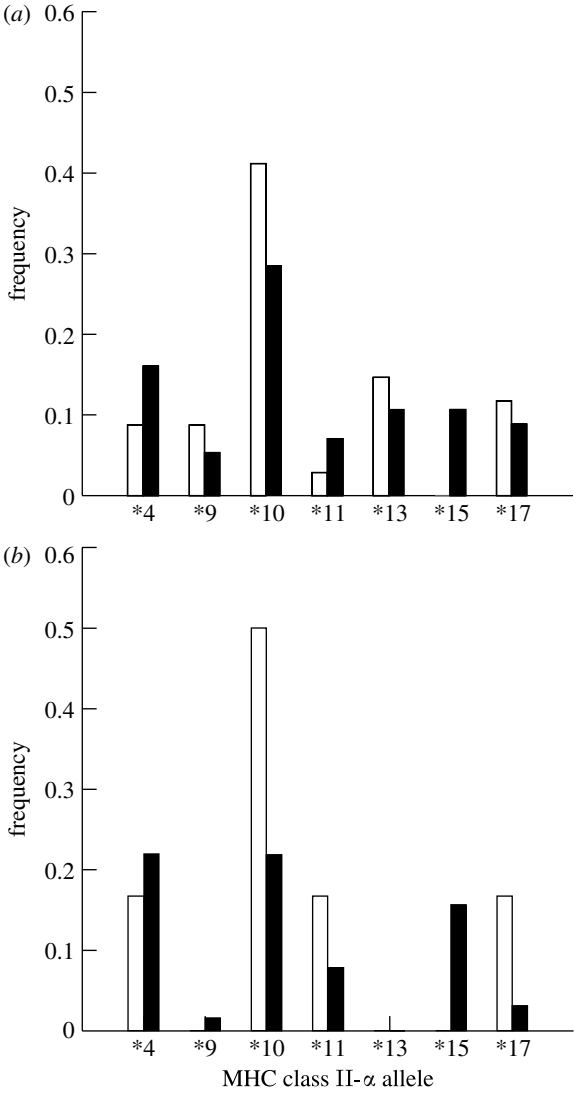

Figure 3. Frequency distribution of MHC class II-α alleles in infected (filled bars) and uninfected (open bars) adult salmon originating from (*a*) natural (wild) and (*b*) artificial crosses (hatchery). Alleles with low prevalence (less than or equal to three individuals) were omitted from analysis. No association was found between specific MHC alleles and parasite resistance in wild ($p=0.603$) or hatchery ($p=0.199$) fish.

(Consuegra & Garcia de Leaniz 2007). On the other hand, evidence for the effect of MHC genotype on parasite resistance in the wild remains controversial (Sommer 2005). Indeed, parasite loads in wild populations of three-spined stickleback seem to be unrelated to MHC genotype (Rauch *et al.* 2006).

We found a much higher abundance of *Anisakis* in the offspring of artificially crossed salmon than that of wild conspecifics, suggesting that in the absence of mate choice salmon may be more susceptible to parasitic infection. Although population levels of MHC genetic diversity were similar for wild and hatchery salmon, the progeny of free mating fish (but not those derived from artificial crosses) were more MHC dissimilar than would be expected by chance. We interpret this as evidence of disassortative mating in the wild, in line with recent work that suggests that the female salmonids may choose males on the basis of MHC dissimilarity (Landry *et al.* 2001; Forsberg *et al.* 2007). Moreover, those individuals resistant to *Anisakis* infection had greater MHC dissimilarity than infected

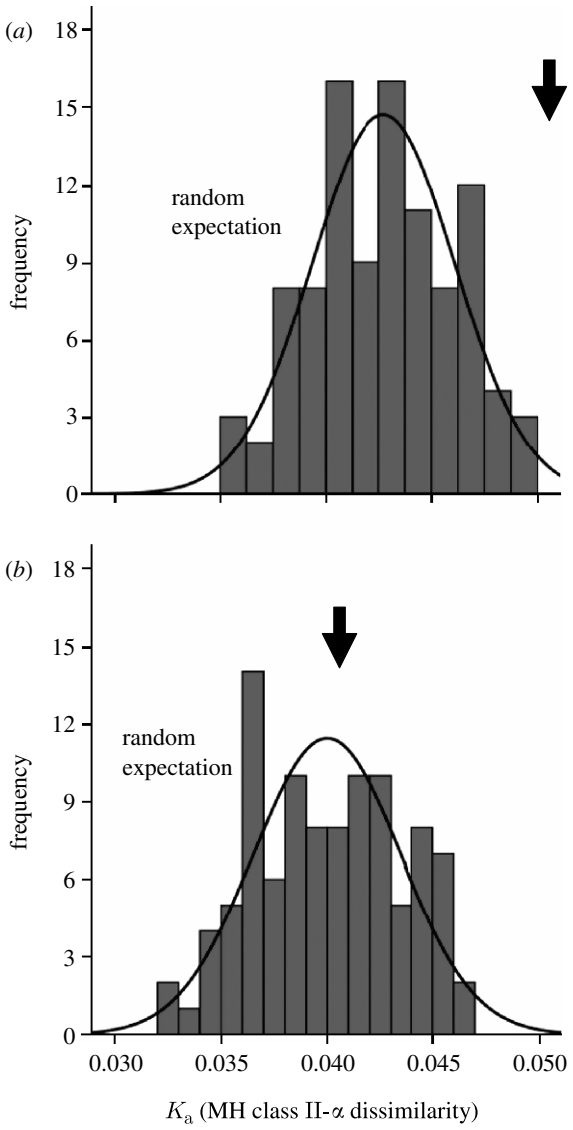

Figure 4. MHC class II-α dissimilarity (K_a; indicated by arrows) of (*a*) free mating (wild) and (*b*) artificially crossed Atlantic salmon (hatchery), compared to random expectations based on 100 bootstrap simulations of MHC allelic frequencies in each group. Unlike artificially bred individuals ($p=0.420$), wild salmon are more MHC dissimilar than would be expected by chance ($p=0.031$), indicative of disassortative mating.

fish, strongly suggesting that the offspring of MHC disassortative matings might benefit from increased resistance to parasitic infection.

We failed to find any association between specific MHC alleles and parasite loads or between heterozygosity and increased parasite resistance. These findings are more consistent with compatible genes than with good genes scenarios, though both hypotheses may not be mutually exclusive (Pitcher & Neff 2006; Reid 2007). For example, conditions favouring mate choice for good genes may alternate with those favouring compatible genes, and some females may select mates for good genes while others may select for compatible genes. Furthermore, the relative importance of additive and non-additive effects on fitness may vary over the course of development (Wedekind *et al.* 2001) and, in some mating systems, females may also accrue non-additive genetic benefits from directional mate choice (Reid 2007).

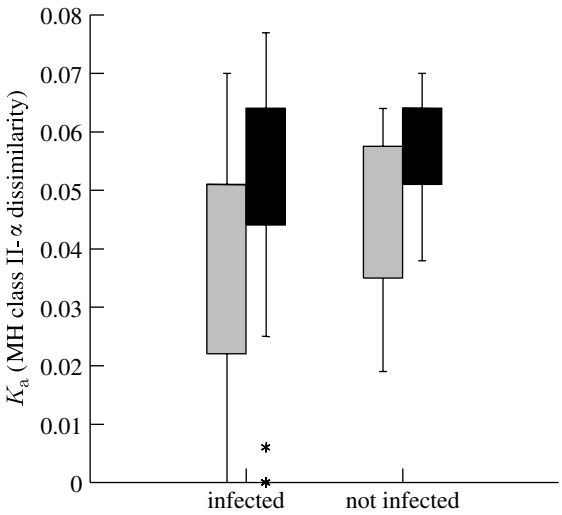

Figure 5. Non-synonymous nucleotide diversity (K_a) in the genotypes of the offspring of free mating (wild, black boxes) and artificially bred (hatchery, grey boxes (no mate choice)) salmon, infected and not infected by *Anisakis*. Box and whisker plots show first and third quartiles (boxes), 90% of values (whiskers), and extreme data points (asterisks). Resistant (non-infected) individuals tend to be more MHC dissimilar than infected ones ($p=0.031$).

We cannot completely disregard the possibility that differences in MHC dissimilarity between hatchery and wild fish might have been caused by non-random sampling of alleles in the hatchery broodstock or by differences in selective pressures during the first months of life. However, the number of different crosses carried out in the captive breeding programme was reasonably large and MHC heterozygosity and allelic richness were the same for hatchery and wild fish. Thus, differences in MHC dissimilarity are most probably due to the effects of mate choice and indicative of disassortative mating among wild fish. It is also unlikely that differences in parasite loads can be attributed to early hatchery rearing (rather than to mate choice), as fish can only become infected with *Anisakis* in the common marine phase during their second or third year of life. Differences in early rearing histories between hatchery and wild fish could have also resulted in temporal or spatial differences in patterns of marine migrations, and consequently in differential exposure to marine parasites. However, the significant association between MHC dissimilarity and resistance to *Anisakis* is also evident when hatchery fish are excluded from analysis, making this interpretation unlikely.

Field studies carried out in the wild are the only ones that can control for genotype×environment interactions, which are very common in fitness traits in salmonids (Garcia de Leaniz *et al.* 2007), and may confound studies of mate choice and genetic quality (Neff & Pitcher 2005). However, one inevitable shortcoming of natural studies is that mating preferences and indirect genetic benefits must necessarily be inferred from posterior analysis of offspring genotypes (e.g. Forsberg *et al.* 2007). Here, we inferred MHC-mediated mate choice from the analysis of F1 genotypes among returning adults, which could have also reflected MHC-related mortality at any stage of development. We do not rule out the possibility of differential mortality of MHC genotypes, but this would not explain the observed differences in MHC dissimilarity between

the progeny of hatchery and wild fish, or the random distribution of MHC alleles among the offspring of artificially bred salmon.

In conclusion, our results suggest that salmon choose mates on the basis of MHC dissimilarity and that the MHC dissimilarity (rather than heterozygosity or specific combinations of alleles) can make their offspring more resistant to parasite infection. We therefore provide some evidence for a link between disassortative mating and offspring benefits and support the idea that both natural and sexual selection shape the evolution of MHC genes. Although our results do not rule out the possibility of 'MHC optimality' (Wegner *et al.* 2003), we did not specifically test for it as Atlantic salmon express only two MHC class II-α alleles.

There is ample evidence that artificially bred animals have lower fitness than wild conspecifics, and this is often attributed to domestication, reduced genetic variation or behavioural deficits (Lynch & O'Hely 2001). Consequently, considerable efforts are made in captive breeding to make rearing conditions more 'natural' or maximize genetic variation. However, our study indicates that artificial breeding may significantly reduce offspring fitness simply through lack of mate choice, as shown recently by Pitcher & Neff (2007). This can have important implications for conservation programmes (including gene banking) and commercial farming alike, since artificial reproduction that negates sexual selection and mate choice may result in inherently inferior offspring, regardless of population size, rearing conditions or genetic diversity.

This research adhered to legal and ethical requirements of the countries where the research took place (Spain and UK).

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