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Paper:

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1   Original Article

2   **Eco-immunology of fish invasions: the role of MHC variation**

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10   Running title: Eco-immunology, MHC and fish invasions

12   Key words: *Oncorhynchus mykiss*, *Salmo trutta*, biological invasions, MHC, enemy-release,

13   immunogenetics

14

15 **Abstract**

16 The relationship between invaders and the pathogens encountered in their new environment

17 can have a large effect on invasion success. Invaders can become free from their natural

18 pathogens and re-allocate costly immune resources to growth and reproduction, thereby

19 increasing invasion success. Release from enemies and relaxation of selective pressures could

20 render newly founded populations more variable at immune-related genes, such as the Major

21 Histocompatibility Complex (MHC), particularly when they have different origins. Using

22 rainbow and brown trout, two of the world most pervasive invasive fish, we tested the general

23 hypothesis that invaders should display high intra-population immunogenetic diversity and

24 inter-population divergence, due to the interplay between genetic drift and successive waves

25 of genetically divergent introductions. We analysed genetic diversity and signatures of

26 selection at the MHC class II-β immune-related locus. In both species MHC diversity (allelic

27 richness and heterozygosity) for Southern Hemisphere populations was similar to values

28 reported for populations at their native range. However, MHC functional diversity was

29 limited and population immunogenetic structuring weaker than that observed using neutral

30 markers. Depleted MHC functional diversity could reflect a decrease in immune response,

31 immune-related assortative mating or selection for resistance to newly encountered parasites.

32 Given that the role of MHC diversity in the survival of the populations remains unclear,

33 depleted functional diversity of invasive salmonids could compromise their long term

34 persistence. A better understanding of the eco-immunology of invaders may help in

35 managing and preventing the impact of biological invasions, a major cause of loss of

36 biodiversity worldwide.

37

Introduction

Biological invasions are the subject of recent scientific controversies that have important economic and societal implications (Simberloff *et al.* 2013), costing billions to global economies (Pimentel *et al.* 2005). Invasive species are important drivers of ecological change (Strayer *et al.* 2006) and biodiversity decline (Butchart *et al.* 2010), but predicting their impacts has proved elusive. Invasives provide some of the most striking examples of rapid evolution (Buswell *et al.* 2011; Carroll 2007; Hendry *et al.* 2008; Whitney & Gabler 2008), and understanding the basis of establishment success is considered key for their management. Pathogens can hamper the reproduction and development of invaders and constrain their invasive potential (Torchin & Mitchell 2004), therefore potentially influencing the outcome of biological invasions. A better understanding of the eco-immunity of invaders could, therefore, provide useful insights into the drivers of establishment success.

In some cases, invasion success can be explained, by the enemy-release hypothesis (Keane & Crawley 2002). According to this, invaders are liberated from their natural enemies (parasites, pathogens and predators) when they colonize new environments, enabling them to re-allocate costly immune defence resources to growth and reproduction that may facilitate invasions (Lee & Klasing 2004). Some support for this comes from the observation that some populations introduced into new environments are less likely to be infected than native populations (Torchin & Mitchell 2004), although the role of novel pathogens on invaders appears to be more complex than what the simple release hypothesis would suggest (Colautti *et al.* 2004). Indeed, founder effects and bottlenecks, could eventually result in immunogenetic losses, rendering invaders more susceptible to novel parasites (White & Perkins 2012). However, relaxation in parasite selective pressures and successive waves of invaders each bringing different parasites and immunogenetic diversity into recipient ecosystems could result in a rapid divergence of populations. In this sense, aquaculture-

63   mediated introductions, the main cause of aquatic invasions together with shipping (Molnar

64   *et al.* 2008; Naylor *et al.* 2001), provide good opportunities to test the role of

65   immunocompetence on fish invasions, as they tend to consist of multiple introductions from

66   different geographical locations (Consuegra *et al.* 2011). Invasions originating from

67   aquaculture escapes could result in an immune repertoire highly divergent among populations

68   due to the interplay between genetic drift and successive waves of genetically diverse

69   introductions.

70   The genes of the major histocompatibility complex (MHC) are excellent candidates

71   for this study. Central to the immune response, MHC genes encode for proteins that present

72   pathogen-derived antigens to T-cells, initiating the adaptive immune response (Janeway *et al.*

73   2004) and are amongst the most polymorphic and best studied functional genes in vertebrates

74   (Hughes & Yeager 1998). Variation in the residues that bind antigens from pathogens is

75   critical for the effectiveness of the immune response (Hedrick & Kim 2000) and is thought to

76   be maintained by balancing selection driven by pathogens (either through over-dominance or

77   frequency-dependent selection, Doherty & Zinkernagel 1975; Slade & McCallum 1992), but

78   also by mate choice (Apanius *et al.* 1997; Consuegra & Garcia de Leaniz 2008). Parasites

79   seem to be the main agent of selection acting on MHC genes, as suggested by multiples lines

80   of evidence provided by heterozygote advantage (Kurtz *et al.* 2004; Wegner *et al.* 2003),

81   rare-allele advantage (Schwensow *et al.* 2007), the association of individual MHC alleles

82   and/or genotypes with susceptibility to specific pathogens (Bonneaud *et al.* 2006b; Gómez *et*

83   *al.* 2010), and changes in allele frequencies after parasite exposure (Eizaguirre *et al.* 2012).

84   Rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) are two salmonids that

85   have been introduced worldwide for sport fishing and aquaculture (Froese & Pauly 2013;

86   Lowe *et al.* 2000). The two species have non-overlapping native ranges but have converged

87   in many parts of Chilean Patagonia (Correa & Hendry 2012; Young *et al.* 2010), where they

4

88   have established and tend to have contrasting introduction histories and dispersal patterns

89   (Young *et al.* 2010). In Chile, rainbow and brown trout were originally introduced for

90   recreational purposes in 1905, most likely as imported ova from US, Germany and England,

91   although additional sources cannot be completely ruled out (Basulto 2003; Wetzlar 1979).

92   Both species were then shipped from Chile to the Falkland Islands between 1936 and 1947,

93   although only brown trout survived and founded self-sustained populations (Arrowsmith and

94   Pentelow 1965). Brown trout displays a narrower geographic range than rainbow trout in

95   Chile but has a stronger impact on native fishes (Young *et al.* 2010; Correa & Hendry 2012),

96   having dispersed mostly through stocking and natural colonization (Gajardo & Laikre 2003;

97   Garcia de Leaniz *et al.* 2010). In contrast, rainbow trout is only present in Chile, where its

98   spread has been facilitated by massive escapes of farmed fish since the 1990s, following the

99   rapid expansion of the Chilean salmon industry (Gajardo & Laikre 2003; Garcia de Leaniz *et*

100 *al.* 2010; Consuegra *et al.* 2011). Here we examined patterns of neutral (microsatellites) and

101 functional (MHC class II-β) genetic diversity in this two ecologically similar invasive

102 salmonids with different modes of dispersal in the Southern Hemisphere to test the general

103 hypothesis that enemy release would results in high immunogenetic diversity and population

104 divergence, particularly in the case of rainbow trout aided by secondary releases.

105

Material and Methods

Fish sampling and laboratory procedures

We analysed a fragment of 254 bp of the exon 2 of the MHC class II-β gene, containing most of the peptide binding region (PBR) in 151 brown trout from six rivers in Chile and three rivers in the Falkland Islands (Figure 1; Table 1) using the primers CL007 (Landry *et al.* 2001) and AL1002 (Olsen *et al.* 1998). Approximately 50 ng of extracted DNA were used in 20 μL PCR mixes containing 0.2 μM of each primer, 0.25 mM dNTPs, 0.5 U of *Taq* DNA polymerase (Bioline, London, UK), 1x buffer and 2.5 mM MgCl$_2$. Thermal conditions consisted of 5 min initial denaturation cycle (94ºC) followed by 35 cycles of 1 min at 94ºC, 1 min at 57ºC, 1 min at 72ºC and a final extension cycle of 10 min at 72ºC. Amplified fragments were directly sequenced using the same PCR primers and resolved in a 3130 automated sequencer (Applied Biosystems). Resulting sequences were aligned using BioEdit 7.0.5.3 (Hall 1999) and compared with previously described brown trout sequences retrieved from GeneBank, as in Consuegra *et al.* (2008), in order to assign alleles. Sequences that had not been previously described were cloned using the TOPO TA Cloning® Kit for Sequencing (Invitrogen) and six clones per individual were selected for forward and reverse sequencing. Only alleles identified in at least 2 independent PCRs were considered for subsequent analyses, these are alleles that appeared in 2 independent PCRs from the same individual or in the independent PCRs of at least 2 individuals. We compared MHC variability in brown trout with that of a 237 base pair fragment of the exon 2 of the MHC class II-β previously amplified in 208 rainbow trout from 10 populations (Figure 1; Table 1) as detailed in (Monzón-Argüello *et al.* 2013). Microsatellite data previously published for both species were used as a baseline for comparisons between neutral (microsatellites) and functional markers (MHC) (7 and 14 microsatellites for rainbow trout and brown trout respectively, Consuegra et al 2011, Monzon-Arguello et al., under revision and stored in Figshare under

6

131 DOI http://dx.doi.org/10.6084/m9.figshare.953191). Rainbow trout had been classified as farm

132 escapees, naturalised or hybrids based on their microsatellite genotypes (Consuegra *et al.*

133 2011).

134

135 *MHC class II-β variability and tests for selection*

136 Observed heterozygosity (H_o) and unbiased expected heterozygosity (H_e) were assessed using

137 Genetix 4.05 (Belkhir *et al.* 2004). Deviations from Hardy-Weinberg (HW) equilibrium

138 following sequential Bonferroni correction (Rice 1989) were estimated using Arlequin 3.5

139 (Excoffier & Lischer 2010). The Ewens-Watterson homozygosity test of neutrality (Ewens

140 1972; Watterson 1978) with Slatkin's exact *P* values (Slatkin 1994; Slatkin 1996) was used to

141 assess deviations from the hypothesis of neutral selection at the MHC locus. The relationship

142 between population diversity (*AR*- allelic richness and *Ho*) at MHC and neutral

143 microsatellites was investigated by using the Pearson correlation coefficient, after testing for

144 normality using SPSS v.19. We analysed the correlation of genetic distance between

145 populations, measured as paiwise F_{ST}, between microsatellites and MHC using a Mantel test

146 implemented in Arlequin. A Mantel test was also used to analyse isolation by distance (IBD).

147 In both species, *AR* and population differentiation (F_{ST}) was determined using FSTAT 2.9.3

148 (Goudet 1995). To investigate population structure, we used the model-based clustering

149 method implemented in STRUCTURE 2.3.3 (Pritchard *et al.* 2000). For each K (1-10), we

150 computed 10 iterations with a burn-in of 20,000 and 80,000 MCMC replicates using the

151 admixture model with allele frequencies correlated. To assess the most likely number of

152 clusters, we calculated ΔK following (Evanno *et al.* 2005).

153 We estimated dissimilarity between MHC class II-β alleles within individuals using

154 the number of non-synonymous substitutions per non-synonymous site (K_a) in DnaSP 5.10

155 (Librado & Rozas 2009) and population observed K_a was compared against random K_a,

156 calculated as in (Consuegra & Garcia de Leaniz 2008). Differences in mean K_a between

157 populations were compared using one-way ANOVA.

158 Evidence for selection was assessed by using three different codon-based maximum

159 likelihood approaches. CODEML implemented in PAML v4.6 (Yang 2007), was used to

160 estimate ω, which is the ratio of the non-synonymous (d_N) to synonymous substitutions (d_S)

161 over the entire set of sequences. We compared models that only consider neutral and

162 deleterious non-synonymous mutations (M1 and M7) with models that allow for selection

163 (M2, M3 and M8). Nested models (M8 and M7; M2, M3 and M1) were compared with a

164 likelihood ratio test (LRT) (Yang & Swanson 2002) and an Akaike Information Criterion

165 (AIC) was used to compare non-nested models. Maximum likelihood trees for the analysis

166 were built using DNAML from PHYLIP (Felsenstein 1995). A Bayesian approach

167 implemented in CODEML was used to identify residues under positive selection in the β

168 domain and sites with a posterior probability > 95% were considered to be positively selected

169 under the model that best fitted the data. In addition, we employed two recent methods

170 implemented in HyPhy (http://www.datamonkey.org/) (Wayne *et al.* 2010), the Mixed

171 Effects Model of Evolution (MEME) (Murrell *et al.* 2012) which detects both episodic and

172 pervasive positive selection at individual sites, and the Fast Unbiased Bayesian

173 Approximation (FUBAR), that detects positive selection under a model that allows site- to-

174 site rate variation (Murrell *et al.* 2013). We only considered sites that attained a significance

175 level of 0.05 in MEME and 0.95 in FURBAR. In order to avoid the potential confounding

176 effect of recombination (Shriner *et al.* 2003), we first examined the presence of intragenic

177 recombination using the GARD analysis also implemented in DataMonkey.

178 Changes in the amino acid sequence of the MHC binding pockets can alter their

179 binding properties and affect the range of pathogen peptides an individual can respond to

180 (Schwensow *et al.* 2007). In order to take this into account, MHC class II-β sequences were

181 clustered into supertypes based on the amino-acid-sequence-properties of all positively

182 selected sites (PSS) as detailed in Ellison *et al.* (2012), based on the procedure of

183 Doytchinova *et al.* (2005)

184

185 **Results**

186 *MHC variability*

187 A total of 40 MHC class II-β alleles were identified in brown trout (19 in Chile and 26 in the

188 Falklands; Figure S1; Table 1), 33 of which represent novel sequences (GenBank Accession

189 No. JX646900 – JX646932). After sequential Bonferroni correction, the MHC class II-β

190 locus deviated significantly from HW expectations in only one population, Estancia Brook

191 ($H_o = 0.810$; $H_e = 0.965$; $P = 0.002$; Table 1). The Ewens-Watterson test following sequential

192 Bonferroni correction rejected the null hypothesis of neutrality only in Estancia Brook

193 (Falkland Islands) (Slatkin exact $P = 0.005$). All rainbow trout populations were in HW

194 equilibrium. There were no significant differences in MHC *AR* or H_o between rainbow and

195 brown trout ($P = 0.238$ and $P = 0.158$, respectively). Non-significant associations between

196 spatial and MHC genetic distances appear inconsistent with a pattern of isolation by distance

197 in both species in Chile (brown trout, $P = 0.406$; rainbow trout, $P = 0.377$). In contrast, there

198 was a highly significant correlation between genetic distance at microsatellite loci and at the

199 MHC class II-β locus in Chilean populations (brown trout, r = 0.676, $P = 0.023$; rainbow

200 trout, r = 0.492; $P = 0.010$).

201 Population differentiation in Chile (F_{ST}) was higher among brown trout than among

202 rainbow trout populations for the MHC class II-β locus (brown trout $F_{ST} = 0.084$ versus

203 rainbow trout $F_{ST} = 0.070$; $P < 0.001$). There was a significant degree of population

204 differentiation among all Chilean and Falkland brown trout populations (Fst = 0.181, $P <$

205   0.001), with brown trout populations within the Falklands displaying the highest level of

206   differentiation ($F_{ST} = 0.194$, $P < 0.001$).

207   STRUCTURE analyses indicated that Chilean rainbow trout were fairly uniform in

208   MHC genotype, with no evidence of population structuring (Figure S2A). Similarly, there

209   was no obvious structuring within Chilean brown trout populations (Figure S2B), whereas

210   brown trout from the Falklands showed a pronounced differentiation with two of the

211   populations (Finlay Creek and Sarnys Creek) grouping together and the third one (Estancia

212   Brook) more closely associated to the Chilean populations (Figure S3).

213   MHC allelic dissimilarity (measured as the mean rate of non-synonymous

214   substitutions, K_a) was significantly lower than the random expectation in four of the Chilean

215   and two of the Falklands brown trout populations (Chile mean $K_a = 0.098$, n = 103, 95% CI =

216   0.011; Falklands mean $K_a = 0.087$, n = 48, 95% CI = 0.017; $P < 0.010$; Figure 2 and Figure

217   S3). In the remaining ones, K_a values were higher than expected by random in one case

218   (Encanto mean $K_a = 0.134$; 95% CI = 0.019) and non-significantly different from random

219   expectation in two others (Blanco-Enco mean $K_a = 0.123$; 95% CI = 0.026; Estancia Brook

220   mean $K_a = 0.110$; 95% CI = 0.027). The mean rate of dissimilarity (K_a) was not significantly

221   different between Chile and Falklands brown trout populations ($P = 0.461$).

222   Based on a similar fragment size (72 sites in rainbow trout and 81 in brown trout),

223   rainbow trout displayed lower MHC allelic dissimilarity than brown trout populations ($P <$

224   0.001), however, as in most of the brown trout populations, MHC allelic dissimilarity was

225   significantly lower than random expectation in nine of the ten rainbow trout populations

226   (mean $K_a = 0.060$, n = 208, 95% CI = 0.005; Figure 2 and Figure S4), with the exception of

227   river Encanto (mean $K_a = 0.074$; 95% CI = 0.012).

228   Cluster analysis revealed 10 consensus brown trout supertypes (based on amino acid

229   sequences), each possessing between 1 and 6 alleles (Figure 3). Supertypes and their

10

230   bootstrapping values were consistent among all methods used (Figure S5). In general, clusters

231   included private alleles from both Chile and Falkland Islands, but some supertypes consisted

232   basically of unique alleles from one region (e.g. Supertype 1 comprised mostly private alleles

233   from Falklands while Supertype 3 was mainly made of private alleles from Chile), resulting

234   in significant differences in supertype composition among populations (F_{ST} = 0.168, $P <$

235   0.001). All of the brown trout from two of the populations in the Falklands (Finlay Creek and

236   Sarnys Creek) carried alleles from supertype 2, 5 or both, whereas the third population

237   (Estancia Brook) displayed a higher diversity of supertypes more similar to the Chilean

238   populations. 30% of the individuals carried alleles from the same supertype, 50% of them

239   with both alleles belonging to Supertypes 5 or 7.

240   In rainbow trout, cluster analysis revealed 6 consensus supertypes, each possessing

241   between 2 and 9 alleles (Figure 4). Supertypes and their bootstrapping values were also

242   consistent among all methods used (Figure S6) and the distribution of supertypes in rainbow

243   trout differed significantly among populations (F_{ST} = 0.040; $P <$ 0.001). As for brown trout,

244   32% of the individuals carried alleles from the same supertype, with a clear predominance of

245   Supertype 4 among those (65%).

246

247   *Signatures of selection*

248   Several codons of the MHC class II-β locus were identified as being under positive selection

249   using three different methods. GARD identified breaking points in codon 65 of the brown

250   trout and 103 of rainbow trout. In Chilean brown trout, maximum likelihood models that

251   allow for positive selection fitted the data significantly better than those that assume only

252   neutral or conserved mutations (Table S1A). AIC suggested that model M2, which allows for

253   positive selection, fitted the data better than the rest of models and identified 21% of sites as

254   being under positive selection (ω = 7.72). All three methods were coincident in identifying

255   three sites under selection in brown trout 66, 77, 80 in Chile (Table 2A). In the Falklands

256   brown trout, M3 model, which assumes three site classes, fitted the data significantly better

257   than the rest (Table S1B). In this case the three methods were coincident in identifying five

258   sites under selection (codon 8, 20, 74, 77, 80). None of these codons was identified as a

259   potential recombination breakpoint and two of them were coincident with those identified in

260   Chilean brown trout (Table 2B).

261   In rainbow trout, the M3 model fitted the data significantly better than the others

262   (Table S1C). Estimates from M3 identified 16% of the sites as being under positive selection

263   in the sequences (ω = 22.56). Codons 35, 47 and 53 (Table 2C), were identified by all

264   methods as being under selection.

265

Discussion

Species introduced into novel environments are often free from their natural pathogens fairly rapidly (Mitchell & Power 2003; Torchin *et al.* 2003), and it usually takes a longer period of time for new pathogens to become established (Lee & Klasing 2004). During the initial invasion stages, hence, invaders can benefit from lower pathogen loads compared to those of conspecifics living within the natural range (Cornell & Hawkins 1993; Kennedy & Pojmanska 1996). We thus hypothesized that successful invaders would display high immunogenetic diversity at genes related to the cell-mediated response (such as the MHC genes) and, due to the combined effects of founder effects and/or potential new waves of invaders, high population differentiation. We tested this hypothesis by comparing MHC class II-β genetic diversity in two co-occurring invasive salmonids from the Southern Patagonia and the Falklands (brown trout and rainbow trout), which are ecologically similar but have different introduction histories. We found levels of MHC class II-β variation for brown and rainbow trout similar or greater than those reported for natural populations at their native range (e.g. Aguilar & Garza 2006). Multiple introductions from several sources might have been able to overcome the potential effects of founder events by introducing new genetic diversity (Consuegra *et al.* 2011; Monzón-Argüello *et al.* 2013). However, we also found evidence of lower diversity than expected by random in most populations of both species at the functional level (measured as amino acid similarity between alleles), potentially caused by a decrease in diversity related to the immune reponse. In theory, successful invaders could display reduced immune activity, in particular that associated with systemic inflammation (including MHC mediated T-cell immunity), and reallocate costly energy resources to other processes such as growth and reproduction (Lee & Klasing 2004). Moreover, at least 30% of individuals of both species possesed alleles belonging to the same supertype, with one

13

290 supertype being clearly predominant in rainbow trout, and two supertypes being predominant

291 in brown trout.

292   Low allelic dissimilarity could also be indicative of assortative MHC-mating (i.e.

293 reproductive pairing of individuals genetically more similar at the MHC class II-β locus than

294 would be expected by random mating) or reflect selective pressures of new pathogens

295 encountered in the new environment. Assortative mating can play a role in sympatric

296 speciation, contributing to pre-mating isolation, and also in the genetic isolation of

297 populations when they come into secondary contact (Bolnick & Kirkpatrick 2012). MHC-

298 related disassortative mating has been observed in a number of species, including house

299 mouse, humans and salmonids (Consuegra& Garcia de Leaniz 2008; Mays Jr& Hill 2004;

300 Penn& Potts 1998), although the difference between diassortative mating and mating for

301 heterozygosity does not seem completely clear (Bonneaud *et al.* 2006a; Roberts *et al.* 2006).

302 In contrast, examples of MHC-mediated assortative mating are less abundant (Roberts *et al.*

303 2005) and none of them in salmonids. Testing for assortative mating was outside the remit of

304 this study and would require experimental evidence but in any case, low dissimilarity seems

305 to contrast with most MHC studies where allele dissimilarity has been commonly identified

306 as a signature of balancing selection (Bernatchez & Landry 2003).

307   We found little evidence of population structuring among Chilean brown trout or

308 rainbow trout in relation to MHC. These results contrast with the high level of structuring

309 previously observed within the same populations using neutral markers (microsatellites): very

310 admixed rainbow trout populations (Consuegra *et al.* 2011) and very structured and

311 differentiated brown trout populations (Monzon-Arguello *et al*. in review). Although the

312 difference could be the result of using a single MHC marker, using the same marker we still

313 observed a clear structuring between brown trout populations from the Falklands and Chile,

314 and among the three populations from the Falklands. For both species, genetic differentiation

at the MHC class II-β gene was correlated with neutral F_{ST}, suggesting a role of neutral evolutionary processes in current populations. However, there was also evidence of selection acting on the PBR of the MHC class II-β in both species when rates of non-synonymous versus synonymous substitutions were considered, and a number of sites appear to be clearly under selection using three different methods. These signatures were fairly consistent between geographical regions in the case of brown trout, suggesting that they may correspond to signatures of selection generated in the original populations before they were introduced in the Southern Hemisphere. This could be because significant d_N to d_S ratios take a long time to accumulate but they may also take an equally long time to disappear in the absence of selection, not necessarily reflecting the effect of current selective pressures (Garrigan & Hedrick 2003).

Finally, we found very uniform patterns of MHC distribution in both species, contrasting with the differences found for neutral markers, where brown trout populations were highly structured whereas rainbow trout displayed high levels of admixture (Consuegra *et al.* 2011; Monzon-Arguello *et al.* under review). This pattern could be explained by the purging of MHC alleles present in fish farms after rainbow trout escape into the wild, as suggested by our previous analyses (Monzón-Argüello *et al.* 2013). Thus, we did not find evidence of an effect of new invasion waves, in the form of aquaculture releases, in the immunogenetic structure of the rainbow trout populations. Instead, we found a similar pattern of reduced functional MHC diversity in both trout species.

The eco-immunology of invasions is an emerging field in need of empirical studies that consider the genetic aspects underlying the immune response, particularly considering the overarching influence of founder effects for the successful establishment of invaders (White & Perkins 2012). We analysed the diversity of MHC class II-β in two contrasting salmonid invaders introduced into the Southern Hemisphere, and potentially liberated of their

340 natural enemies. MHC class II-β genetic diversity did not appear to be reduced in terms of

341 allelic richness or heterozygosity in any of the two species. However, we found evidence of

342 low functional MHC diversity (measured as amino acid dissimilarity) in most populations of

343 both species, high percentage of individuals with two alleles from the same functionally

344 similar supertype and lower population genetic structuring than that observed at neutral

345 markers, suggesting a potential reduction in MHC functional diversity that could reflect

346 either a decrease in cell-immune response, assortative mating or new pathogen pressures, all

347 hypotheses that deserve further experimental studies. The relationship between MHC

348 diversity and the long term persistence of small populations is unclear (Radwan *et al.* 2010).

349 While some species seem to thrive even after severe bottlenecks have depleted their MHC

350 diversity, it could be that these species only represent the rare examples that survived despite

351 of the loss of MHC variation (Radwan *et al.* 2010). Given the potential importance of host-

352 parasite relationships for the establishment and long-term persistence of invasive species we

353 suggest that a better understanding of the eco-immunology of invaders may help in managing

354 and preventing the impact of biological invasions.

Data accessibility

Raw data on microsatellites has been stored in Figshre (http://dx.doi.org/10.6084/m9.figshare.953191) and will be made accessible though Figshare when the paper is accepted.

372 **Figure captions**

373 **Figure 1.** Sampling locations of rainbow trout (*Oncorhynchus mykiss*) and brown trout

374 (*Salmo trutta*) populations in Chile and the Falkland Islands. Open and closed circles

375 represent rainbow trout and brown trout populations, respectively, while stars represent rivers

376 sampled for both species.

377 **Figure 2.** MHC class II-β dissimilarity (K_a; indicated by arrows) of (A) Chilean brown trout

378 (B) Falklands brown trout and (C) Chilean Rainbow trout, compared to random expectations

379 based on 100 permutations of MHC allelic frequencies in each group.

380 **Figure 3.** Phenetic tree based on a cluster analysis (Ward's algorithm) defining MHC brown

381 trout Supertypes. * and ** show private alleles in Chile and the Falkland Islands populations,

382 respectively.

383 **Figure 4.** Phenetic tree based on a cluster analysis (Ward's algorithm) defining MHC

384 rainbow trout supertypes.

385

386   **Figure 1.**

387

388

389   **Figure 2.**
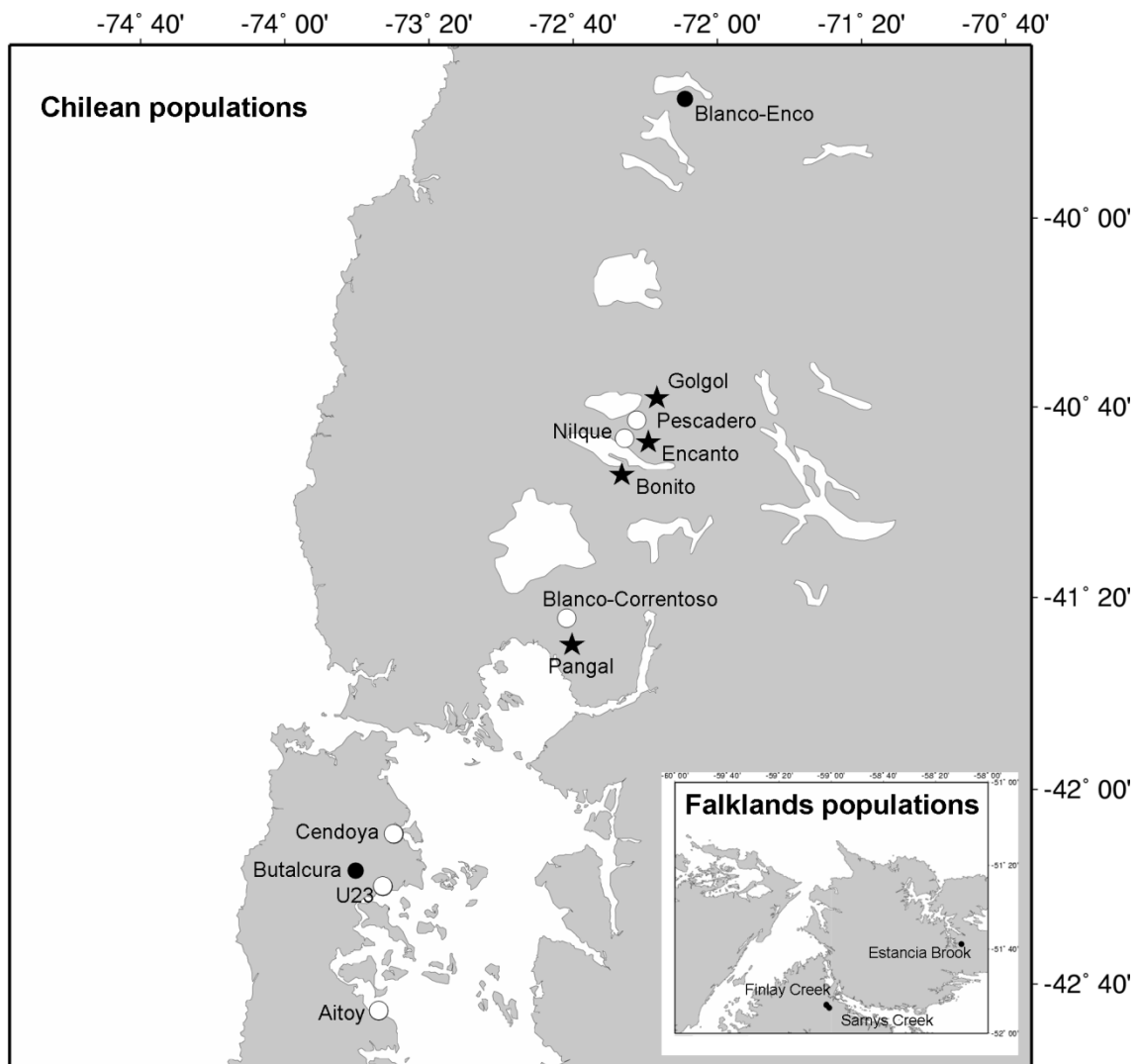
(A) Chilean Brown Trout

(B) Falklands Brown Trout

(C) Chilean Rainbow Trout

390

391

392   **Figure 3.**

393
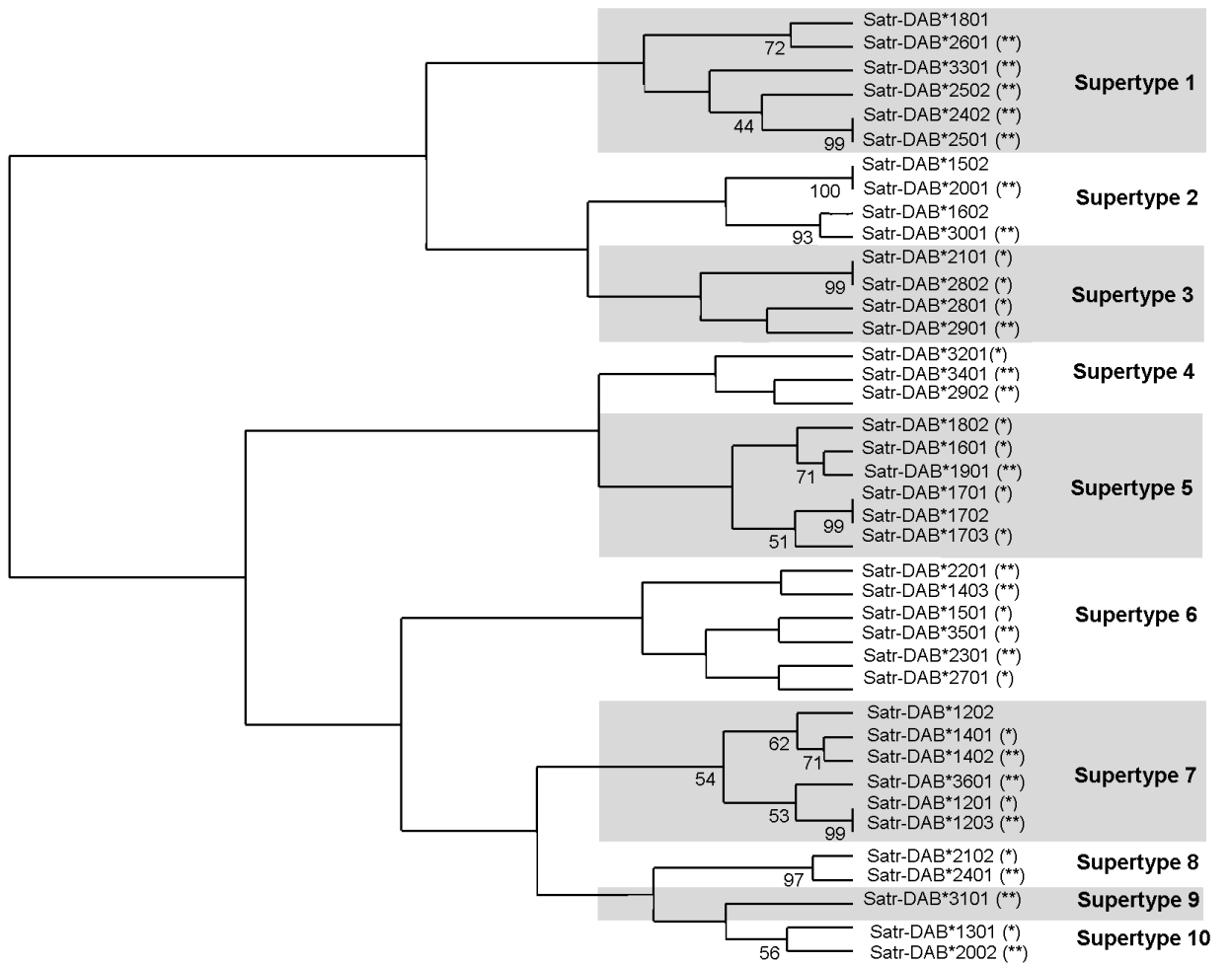
394   **Figure 4.**

395

396

23
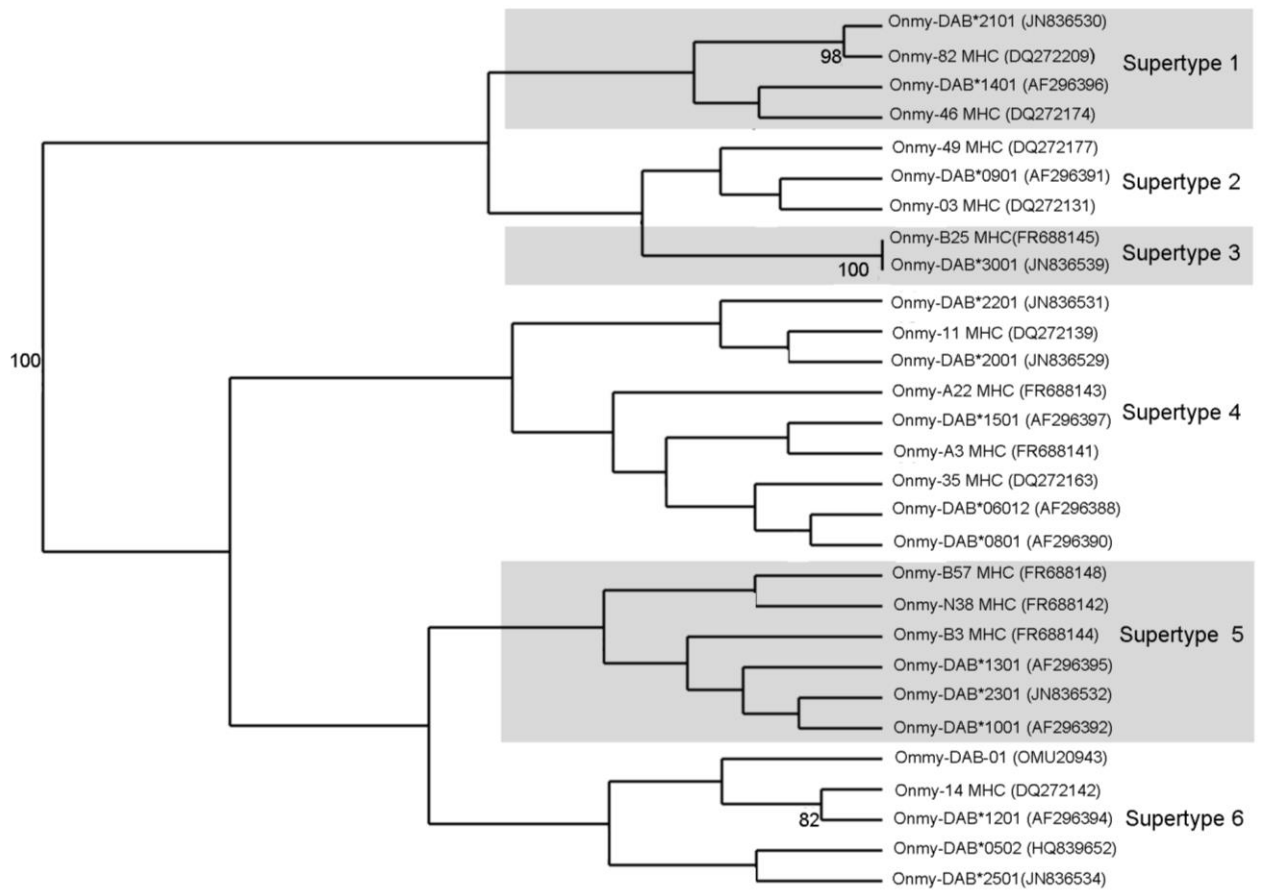
397 **Table 1.** Diversity indices for 9 brown trout and 10 rainbow trout populations at neutral microsatellite (Micr
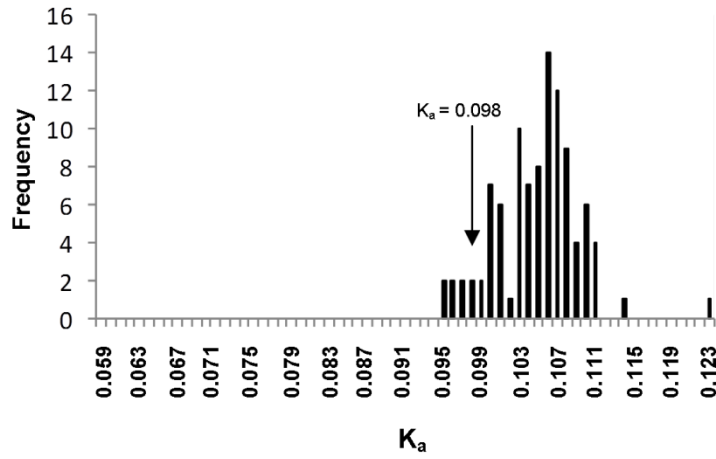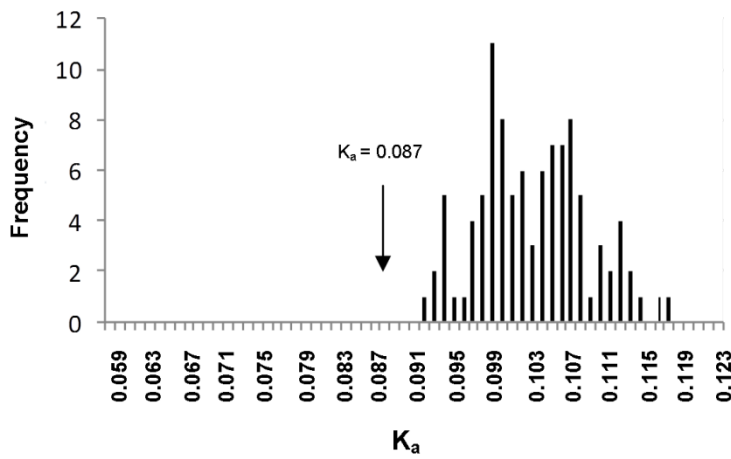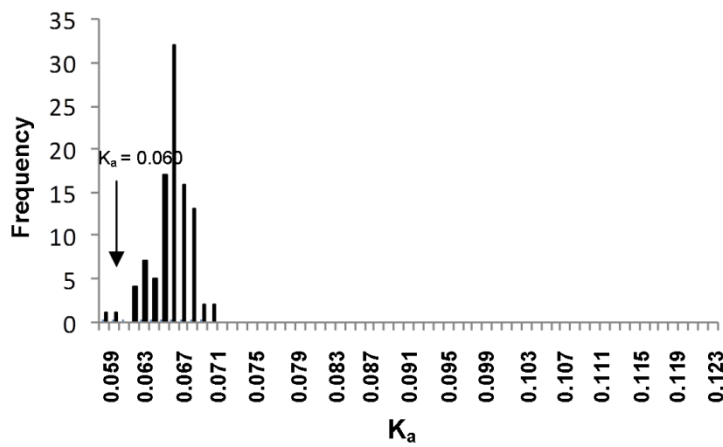
398 sample size; *K*, number of observed alleles; *AR*, allelic richness (based on 7 diploid individuals); H_o, obser

399 heterozygosity and *J'*, evenness population admixture index.

Species		Population	Marker	N	K	*AR*	*H*
Brown Trout	Chile	Golgol	Microsat/MHC	21	5.571/14	4.347/8.306	0.
		Butalcura	Microsat/MHC	22	4.643/11	3.895/6.241	0.
		Blanco-Enco	Microsat/MHC	19	5.071/8	4.071/6.632	0.
		Pangal	Microsat/MHC	23	4.143/8	3.551/6.161	0.
		Encanto	Microsat/MHC	21	5.214/7	4.134/5.925	0.
		Bonito	Microsat/MHC	20	5.214/7	4.316/6.221	0.
	Falklands	Estancia Brook	Microsat/MHC	23	7.929/21	5.634/11.137	0.
		Finlay Creek	Microsat/MHC	23	2.786/4	2.446/3.084	0.
		Sarnys Creek	Microsat/MHC	15	3.143/6	2.664/6.000	0.
Rainbow Trout	Chile	Encanto	Microsat/MHC	23	7.429/11	6.412/9.118	0.
		Nilque	Microsat/MHC	23	6.714/12	6.135/9.255	0.
		Pescadero	Microsat/MHC	24	7.429/16	6.455/12.752	0.
		Blanco-Correntoso	Microsat/MHC	20	6.875/11	6.253/9.422	0.
		U23	Microsat/MHC	17	7.286/14	7.023/12.410	0.

24

Aitoy	Microsat/MHC	16	7.714/12	7.478/11.282	0.
Pangal	Microsat/MHC	16	6.714/14	6.425/12.69	0.
Bonito	Microsat/MHC	24	7.714/12	6.592/9.197	0.
Golgol	Microsat/MHC	24	7.143/9	5.906/7.409	0.
Cendoya	Microsat/MHC	24	4.857/5	4.283/4.988	0.

400

401 **Table 2.** Results from three different codon-based maximum likelihood approaches; Mixed Effects Model of

402 Approximation (FUBAR) and the model in CODEML that best fit the data to estimate positive selected sites

403 brown trout (A), Falklands brown trout (B) and Chilean rainbow trout (C). In bold are the sites identified a

404 methods.

Model	Positively selected sites
(A) Brown Trout Chile	
MEME	52, 57, 63, **66, 77, 80**
FURBAR	8, 12, 31, 33, **66**, 74, **77, 80**
M2 (Positive selection)	4Y**, 6R*, 8A**, 22L*, 31A**, 33Y**, 52K*, 63I**, **66Q**,** 74Y**, **77P**, 80D**,** 81I**
(B) Brown Trout Falklands	
MEME	**8, 20,** 27, 52, 66, **74, 77, 80**, 82
FURBAR	**8, 20,** 31, 34, 52, **74, 77, 80**, 81, 82
M3 (Discrete)	4E**, 5Q**, 6V**, 7V**, **8R**,** 9Q**, 11R** ,12F**, 19G**, **20I**,** 22F**, 24D**, 27V* 34V**, 43Y**, 49H**, 52K**, 57W**, 61G**, 62P**, 63E**, 66Q**, 67E**, 68L**, 70E* **80A**,** 81I**, 82D**
(C) Rainbow Trout Chile	
MEME	8, **35, 47, 53**
FURBAR	4, 6, 17, 27, **35, 47, 53**, 58, 61, 66
M3 (Discrete)	4I**, 6F**, 7I*, 8D**, 11V**, 14K**, 15V**, 17H**, 18I**, 27Y**, **35V***, 41W**, **47L**** 58Y**, 61H**, 63A**, 64D**, 65I**, 66Y**

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