Original Article

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

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Abstract

The relationship between invaders and the pathogens encountered in their new environment can have a large effect on invasion success. Invaders can become free from their natural pathogens and re-allocate costly immune resources to growth and reproduction, thereby increasing invasion success. Release from enemies and relaxation of selective pressures could render newly founded populations more variable at immune-related genes, such as the Major Histocompatibility Complex (MHC), particularly when they have different origins. Using rainbow and brown trout, two of the world most pervasive invasive fish, we tested the general hypothesis that invaders should display high intra-population immunogenetic diversity and inter-population divergence, due to the interplay between genetic drift and successive waves of genetically divergent introductions. We analysed genetic diversity and signatures of selection at the MHC class II-β immune-related locus. In both species MHC diversity (allelic richness and heterozygosity) for Southern Hemisphere populations was similar to values reported for populations at their native range. However, MHC functional diversity was limited and population immunogenetic structuring weaker than that observed using neutral markers. Depleted MHC functional diversity could reflect a decrease in immune response, immune-related assortative mating or selection for resistance to newly encountered parasites. Given that the role of MHC diversity in the survival of the populations remains unclear, depleted functional diversity of invasive salmonids could compromise their long term persistence. A better understanding of the eco-immunology of invaders may help in managing and preventing the impact of biological invasions, a major cause of loss of biodiversity worldwide.

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

have established and tend to have contrasting introduction histories and dispersal patterns (Young *et al.* 2010). In Chile, rainbow and brown trout were originally introduced for recreational purposes in 1905, most likely as imported ova from US, Germany and England, although additional sources cannot be completely ruled out (Basulto 2003; Wetzlar 1979). Both species were then shipped from Chile to the Falkland Islands between 1936 and 1947, although only brown trout survived and founded self-sustained populations (Arrowsmith and Pentelow 1965). Brown trout displays a narrower geographic range than rainbow trout in Chile but has a stronger impact on native fishes (Young *et al.* 2010; Correa & Hendry 2012), having dispersed mostly through stocking and natural colonization (Gajardo & Laikre 2003; Garcia de Leaniz *et al.* 2010). In contrast, rainbow trout is only present in Chile, where its spread has been facilitated by massive escapes of farmed fish since the 1990s, following the rapid expansion of the Chilean salmon industry (Gajardo & Laikre 2003; Garcia de Leaniz *et al.* 2010; Consuegra *et al.* 2011). Here we examined patterns of neutral (microsatellites) and functional (MHC class II-β) genetic diversity in this two ecologically similar invasive salmonids with different modes of dispersal in the Southern Hemisphere to test the general hypothesis that enemy release would results in high immunogenetic diversity and population divergence, particularly in the case of rainbow trout aided by secondary releases.

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

DOI http://dx.doi.org/10.6084/m9.figshare.953191). Rainbow trout had been classified as farm escapees, naturalised or hybrids based on their microsatellite genotypes (Consuegra *et al.* 2011).

MHC class II-β variability and tests for selection

Observed heterozygosity (H_o) and unbiased expected heterozygosity (H_e) were assessed using Genetix 4.05 (Belkhir *et al.* 2004). Deviations from Hardy-Weinberg (HW) equilibrium following sequential Bonferroni correction (Rice 1989) were estimated using Arlequin 3.5 (Excoffier & Lischer 2010). The Ewens-Watterson homozygosity test of neutrality (Ewens 1972; Watterson 1978) with Slatkin's exact *P* values (Slatkin 1994; Slatkin 1996) was used to assess deviations from the hypothesis of neutral selection at the MHC locus. The relationship between population diversity (*AR*- allelic richness and *Ho*) at MHC and neutral microsatellites was investigated by using the Pearson correlation coefficient, after testing for normality using SPSS v.19. We analysed the correlation of genetic distance between populations, measured as paiwise F_{ST}, between microsatellites and MHC using a Mantel test implemented in Arlequin. A Mantel test was also used to analyse isolation by distance (IBD). In both species, *AR* and population differentiation (F_{ST}) was determined using FSTAT 2.9.3 (Goudet 1995). To investigate population structure, we used the model-based clustering method implemented in STRUCTURE 2.3.3 (Pritchard *et al.* 2000). For each K (1-10), we computed 10 iterations with a burn-in of 20,000 and 80,000 MCMC replicates using the admixture model with allele frequencies correlated. To assess the most likely number of clusters, we calculated ΔK following (Evanno *et al.* 2005).

We estimated dissimilarity between MHC class II-β alleles within individuals using the number of non-synonymous substitutions per non-synonymous site (K_a) in DnaSP 5.10 (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

clustered into supertypes based on the amino-acid-sequence-properties of all positively selected sites (PSS) as detailed in Ellison *et al.* (2012), based on the procedure of Doytchinova *et al.* (2005)

Results

MHC variability

A total of 40 MHC class II-β alleles were identified in brown trout (19 in Chile and 26 in the Falklands; Figure S1; Table 1), 33 of which represent novel sequences (GenBank Accession No. JX646900 – JX646932). After sequential Bonferroni correction, the MHC class II-β locus deviated significantly from HW expectations in only one population, Estancia Brook (H_o = 0.810; H_e = 0.965; P = 0.002; Table 1). The Ewens-Watterson test following sequential Bonferroni correction rejected the null hypothesis of neutrality only in Estancia Brook (Falkland Islands) (Slatkin exact P = 0.005). All rainbow trout populations were in HW equilibrium. There were no significant differences in MHC *AR* or H_o between rainbow and brown trout (P = 0.238 and P = 0.158, respectively). Non-significant associations between spatial and MHC genetic distances appear inconsistent with a pattern of isolation by distance in both species in Chile (brown trout, P = 0.406; rainbow trout, P = 0.377). In contrast, there was a highly significant correlation between genetic distance at microsatellite loci and at the MHC class II-β locus in Chilean populations (brown trout, r = 0.676, P = 0.023; rainbow trout, r = 0.492; P = 0.010).

Population differentiation in Chile (F_{ST}) was higher among brown trout than among rainbow trout populations for the MHC class II-β locus (brown trout F_{ST} =0.084 versus rainbow trout F_{ST} = 0.070; P < 0.001). There was a significant degree of population 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

bootstrapping values were consistent among all methods used (Figure S5). In general, clusters included private alleles from both Chile and Falkland Islands, but some supertypes consisted basically of unique alleles from one region (e.g. Supertype 1 comprised mostly private alleles from Falklands while Supertype 3 was mainly made of private alleles from Chile), resulting in significant differences in supertype composition among populations (F_{ST} = 0.168, P < 0.001). All of the brown trout from two of the populations in the Falklands (Finlay Creek and Sarnys Creek) carried alleles from supertype 2, 5 or both, whereas the third population (Estancia Brook) displayed a higher diversity of supertypes more similar to the Chilean populations. 30% of the individuals carried alleles from the same supertype, 50% of them with both alleles belonging to Supertypes 5 or 7.

In rainbow trout, cluster analysis revealed 6 consensus supertypes, each possessing between 2 and 9 alleles (Figure 4). Supertypes and their bootstrapping values were also consistent among all methods used (Figure S6) and the distribution of supertypes in rainbow trout differed significantly among populations (F_{ST} = 0.040; P < 0.001). As for brown trout, 32% of the individuals carried alleles from the same supertype, with a clear predominance of Supertype 4 among those (65%).

Signatures of selection

Several codons of the MHC class II-β locus were identified as being under positive selection using three different methods. GARD identified breaking points in codon 65 of the brown trout and 103 of rainbow trout. In Chilean brown trout, maximum likelihood models that allow for positive selection fitted the data significantly better than those that assume only neutral or conserved mutations (Table S1A). AIC suggested that model M2, which allows for positive selection, fitted the data better than the rest of models and identified 21% of sites as being under positive selection (ω = 7.72). All three methods were coincident in identifying

three sites under selection in brown trout 66, 77, 80 in Chile (Table 2A). In the Falklands brown trout, M3 model, which assumes three site classes, fitted the data significantly better than the rest (Table S1B). In this case the three methods were coincident in identifying five sites under selection (codon 8, 20, 74, 77, 80). None of these codons was identified as a potential recombination breakpoint and two of them were coincident with those identified in Chilean brown trout (Table 2B).

In rainbow trout, the M3 model fitted the data significantly better than the others (Table S1C). Estimates from M3 identified 16% of the sites as being under positive selection in the sequences (ω = 22.56). Codons 35, 47 and 53 (Table 2C), were identified by all methods as being under selection.

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

supertype being clearly predominant in rainbow trout, and two supertypes being predominant in brown trout.

Low allelic dissimilarity could also be indicative of assortative MHC-mating (i.e. reproductive pairing of individuals genetically more similar at the MHC class II-β locus than would be expected by random mating) or reflect selective pressures of new pathogens encountered in the new environment. Assortative mating can play a role in sympatric speciation, contributing to pre-mating isolation, and also in the genetic isolation of populations when they come into secondary contact (Bolnick & Kirkpatrick 2012). MHC-related disassortative mating has been observed in a number of species, including house mouse, humans and salmonids (Consuegra& Garcia de Leaniz 2008; Mays Jr& Hill 2004; Penn& Potts 1998), although the difference between diassortative mating and mating for heterozygosity does not seem completely clear (Bonneaud *et al.* 2006a; Roberts *et al.* 2006). In contrast, examples of MHC-mediated assortative mating are less abundant (Roberts *et al.* 2005) and none of them in salmonids. Testing for assortative mating was outside the remit of this study and would require experimental evidence but in any case, low dissimilarity seems to contrast with most MHC studies where allele dissimilarity has been commonly identified as a signature of balancing selection (Bernatchez & Landry 2003).

We found little evidence of population structuring among Chilean brown trout or rainbow trout in relation to MHC. These results contrast with the high level of structuring previously observed within the same populations using neutral markers (microsatellites): very admixed rainbow trout populations (Consuegra *et al.* 2011) and very structured and differentiated brown trout populations (Monzon-Arguello *et al*. in review). Although the difference could be the result of using a single MHC marker, using the same marker we still observed a clear structuring between brown trout populations from the Falklands and Chile, and among the three populations from the Falklands. For both species, genetic differentiation

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

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

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

natural enemies. MHC class II-β genetic diversity did not appear to be reduced in terms of allelic richness or heterozygosity in any of the two species. However, we found evidence of low functional MHC diversity (measured as amino acid dissimilarity) in most populations of both species, high percentage of individuals with two alleles from the same functionally similar supertype and lower population genetic structuring than that observed at neutral markers, suggesting a potential reduction in MHC functional diversity that could reflect either a decrease in cell-immune response, assortative mating or new pathogen pressures, all hypotheses that deserve further experimental studies. The relationship between MHC diversity and the long term persistence of small populations is unclear (Radwan *et al.* 2010). While some species seem to thrive even after severe bottlenecks have depleted their MHC diversity, it could be that these species only represent the rare examples that survived despite of the loss of MHC variation (Radwan *et al.* 2010). Given the potential importance of host-parasite relationships for the establishment and long-term persistence of invasive species we suggest that a better understanding of the eco-immunology of invaders may help in managing and preventing the impact of biological invasions.

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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.

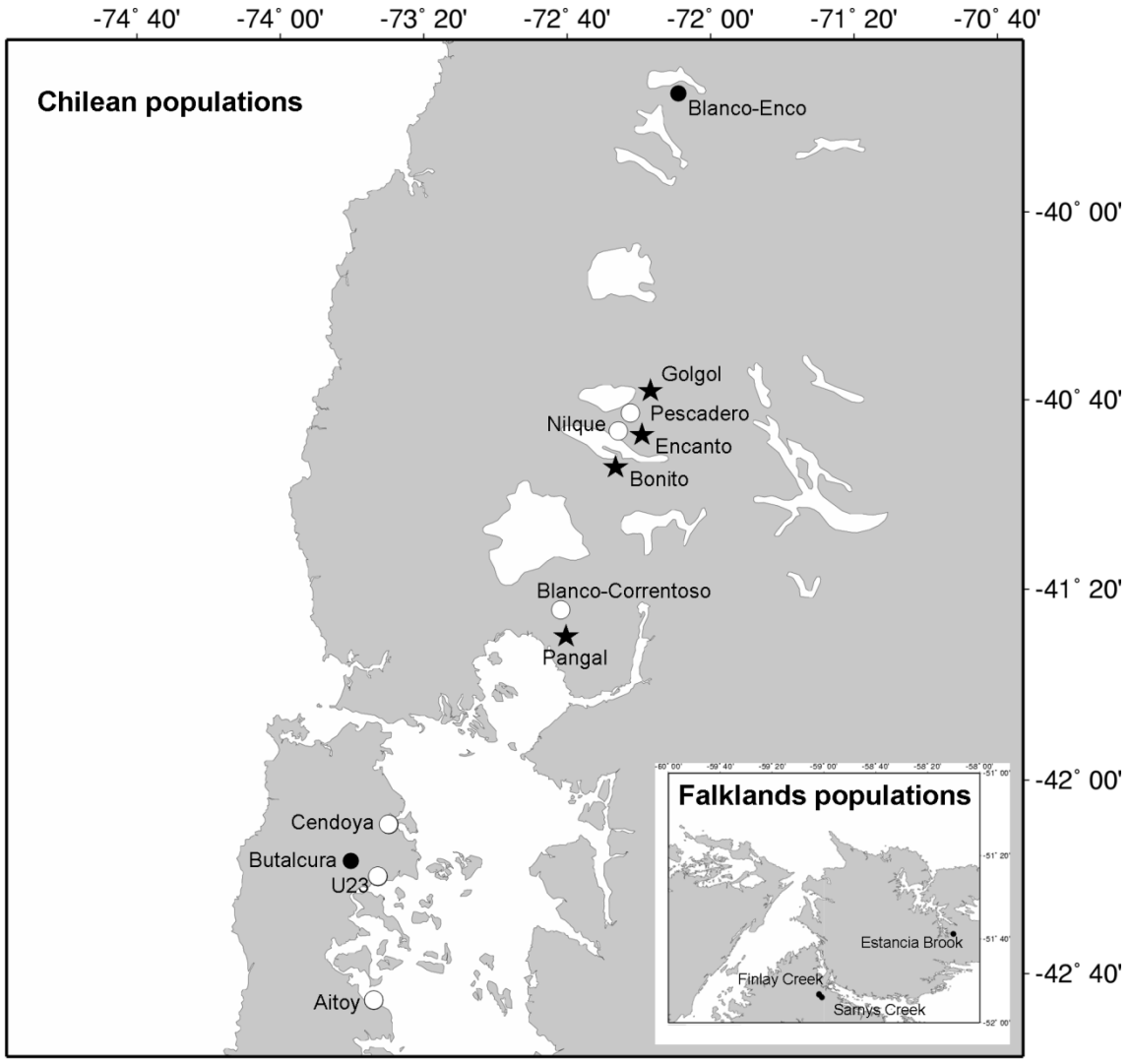
377 **Figure 2.** MHC class II-β dissimilarity (K_a; indicated by arrows) of (A) Chilean brown trout
378 (B) Falklands brown trout and (C) Chilean Rainbow trout, compared to random expectations
379 based on 100 permutations of MHC allelic frequencies in each group.

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

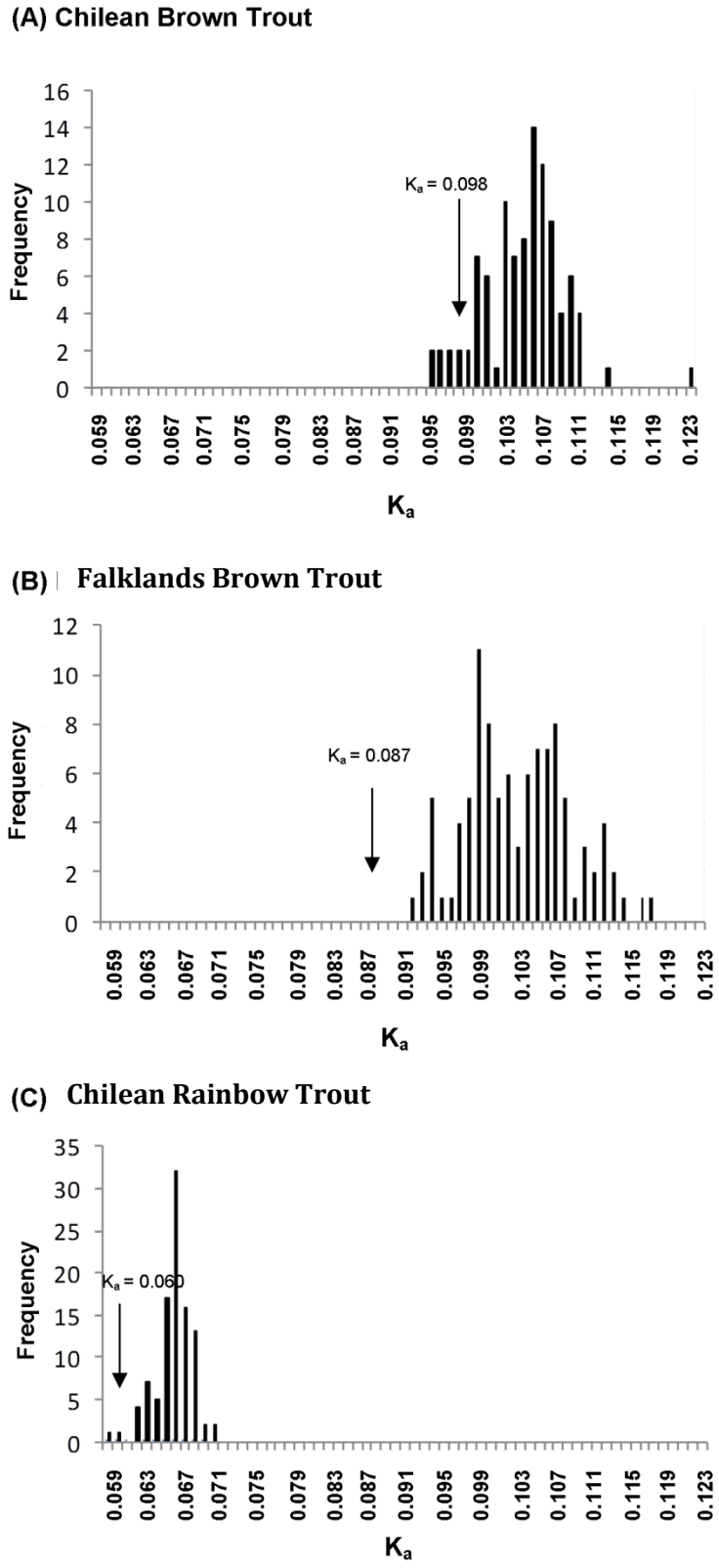
386 **Figure 1.**



387

388

389 **Figure 2.**



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392 **Figure 3.**

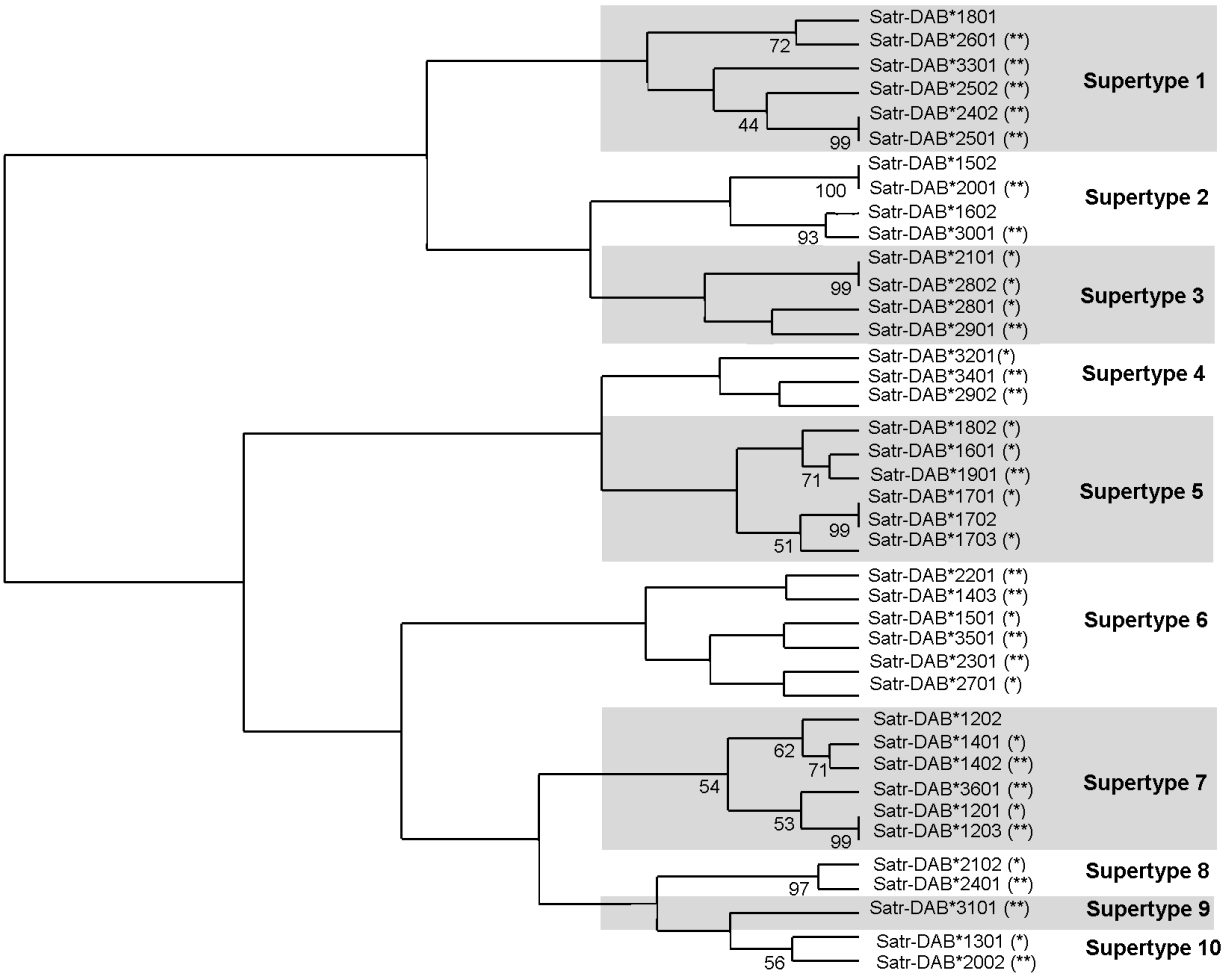

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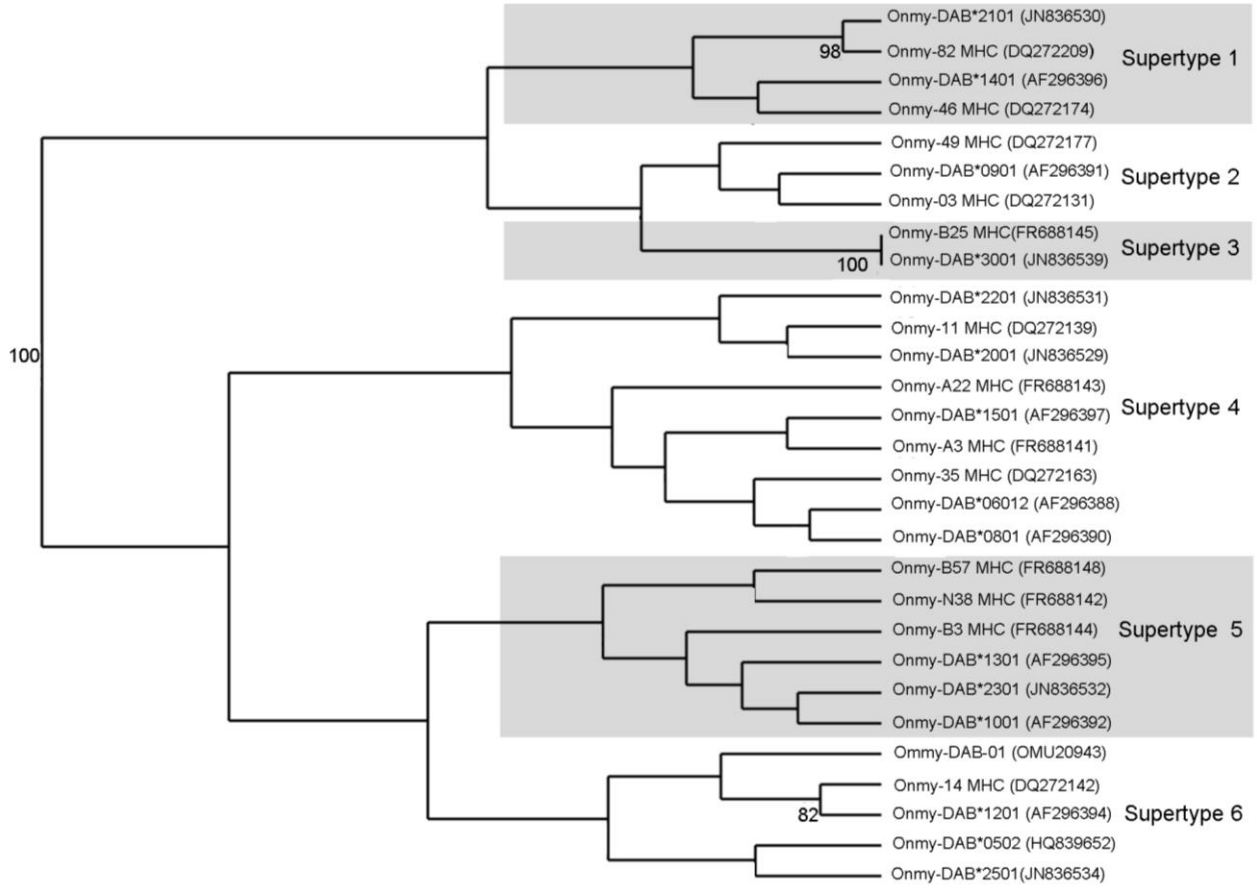
Figure 4.


Onmy-DAB*2101 (JN836530)
98
Onmy-82 MHC (DQ272209)
Onmy-DAB*1401 (AF296396)
Onmy-46 MHC (DQ272174)
Supertype 1
Onmy-49 MHC (DQ272177)
Onmy-DAB*0901 (AF296391)
Onmy-03 MHC (DQ272131)
Supertype 2
Onmy-B25 MHC(FR688145)
100
Onmy-DAB*3001 (JN836539)
Supertype 3
100
Onmy-DAB*2201 (JN836531)
Onmy-11 MHC (DQ272139)
Onmy-DAB*2001 (JN836529)
Onmy-A22 MHC (FR688143)
Onmy-DAB*1501 (AF296397)
Onmy-A3 MHC (FR688141)
Onmy-35 MHC (DQ272163)
Onmy-DAB*06012 (AF296388)
Onmy-DAB*0801 (AF296390)
Supertype 4
Onmy-B57 MHC (FR688148)
Onmy-N38 MHC (FR688142)
Onmy-B3 MHC (FR688144)
Onmy-DAB*1301 (AF296395)
Onmy-DAB*2301 (JN836532)
Onmy-DAB*1001 (AF296392)
Supertype 5
Ommy-DAB-01 (OMU20943)
Onmy-14 MHC (DQ272142)
82
Onmy-DAB*1201 (AF296394)
Onmy-DAB*0502 (HQ839652)
Onmy-DAB*2501(JN836534)
Supertype 6

396

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

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.

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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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References

Aguilar A, Garza JC (2006) A comparison of variability and population structure for major histocompatibility complex and microsatellite loci in California coastal steelhead (Oncorhynchus mykiss Walbaum). Mol Ecol 15: 923-937.

Apanius V, Penn D, Slev PR, Ruff LR, Potts WK (1997) The nature of selection on the major histocompatibility complex. Crit Rev Immunol 17: 179 - 224.

Arrowsmith E, Pentelow F (1965) The introduction of trout and salmon to the Falkland Islands. Salmon and Trout Magazine 174: 119-129.

Basulto S (2003) El largo viaje de los salmones: una crónica olvidada, propagación y cultivo de especies acuáticas en Chile, p. 299. Editorial Maval, Santiago de Chile.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) GENETIX 4.05, logiciel sous Windows TM pour la genetique des populations. Laboratoire Genome, Populations, Universite de Montpellier II, Montpellier, France.

Bernatchez L, Landry C (2003) MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? J Evol Biol 16: 363-377.

Bolnick DI, Kirkpatrick M (2012) The relationship between intraspecific assortative mating and reproductive isolation between divergent populations. Curr Zool 58: 484-492.

Bonneaud C, Chastel O, Federici P, Westerdahl H, Sorci G (2006a) Complex MHC-based mate choice in a wild passerine. Proc R Soc B-Biol Sci 273: 1111 - 1116.

Bonneaud C, Pérez-Tris J, Federici P, Chastel O, Sorci G (2006b) Major Histocompatibility alleles associated with local resistance to malaria in a passerine. Evolution 60: 383-389.

Buswell JM, Moles AT, Hartley S (2011) Is rapid evolution common in introduced plant species? (vol 99, pg 214, 2011). J Ecol 99: 1566-1566.

Butchart SHM, Walpole M, Collen B, et al. (2010) Global Biodiversity: Indicators of Recent Declines. Science 328: 1164-1168.

Cairney M, Taggart JB, Hoyheim B (2000) Characterization of microsatellite and minisatellite loci in Atlantic salmon (Salmo salar L.) and cross-species amplification in other salmonids. Mol Ecol 9: 2175-2178.

Carroll SP (2007) Natives adapting to invasive species: ecology, genes, an the sustainability of conservation. Ecol Res 22: 892-901.

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecol Lett 7: 721-733.

Consuegra S, Garcia de Leaniz C (2008) MHC-mediated mate choice increases parasite resistance in salmon. Proc R Soc B-Biol Sci 275: 1397-1403.

Consuegra S, Phillips N, Gajardo G, Garcia de Leaniz C (2011) Winning the invasion roulette: escapes from fish farms increase admixture and facilitate establishment of non-native rainbow trout. Evolut Appl: 660-671.

Cornell HV, Hawkins BA (1993) Accumulation of Native Parasitoid Species on Introduced Herbivores: A Comparison of Hosts as Natives and Hosts as Invaders. Am Nat 141: 847-865.

Correa C, Hendry AP (2012) Invasive salmonids and lake order interact in the decline of puye grande Galaxias platei in western Patagonia lakes. Ecol Appl 22: 828–842.

Doherty PC, Zinkernagel RM (1975) Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. Nature 256: 50-52.

456 Doytchinova IA, Walshe V, Borrow P, Flower DR (2005) Towards the chemometric
457 dissection of peptide - HLA-A*0201 binding affinity: comparison of local and
458 global QSAR models. Journal of Computer-Aided Molecular Design 19.
459 Eizaguirre C, Lenz TL, Kalbe M, Milinski M (2012) Rapid and adaptive evolution of
460 MHC genes under parasite selection in experimental vertebrate populations. Nat
461 Comm 3: 621.
462 Ellison A, Allainguillaume J, Girdwood S, et al. (2012) Maintaining functional major
463 histocompatibility complex diversity under inbreeding: the case of a selfing
464 vertebrate. Proc R Soc B-Biol Sci 279: 5004-5013.
465 Estoup A, Presa P, Krieg F, Vaiman D, Guyomard R (1993) (CT)(N) and (GT)(N)
466 microsatellites - a new class of genetic-markers for Salmo trutta L (Brown
467 trout). Heredity 71: 488-496.
468 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals
469 using the softwarestructure: a simulation study. Mol Ecol 14: 2611-2620.
470 Ewens WJ (1972) The sampling theory of selectively neutral alleles. Theoret Pop Biol
471 3: 87-112.
472 Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to
473 perform population genetics analyses under Linux and Windows. Mol Ecol
474 Resources 10: 564-567.
475 Felsenstein J (1995) Phylip (Phylogeny Inference Package) Department of Genetics,
476 University of Washington, Seattle.
477 Froese R, Pauly D (2013) FishBase. World Wide Web electronic publication.
478 Gajardo G, Laikre L (2003) Chilean Aquaculture boom Is based on exotic salmon
479 resources: a conservation paradox. Cons Biol 17: 1173-1174.

480 Garcia de Leaniz C, Gajardo G, Consuegra S (2010) From best to pest: changing
481 perspectives on the impact of exotic salmonids in the Southern Hemisphere.
482 System Biodiv 8: 447-459.
483 Garrigan D, Hedrick PW (2003) Detecting adaptive molecular polymorphism: lessons
484 from the MHC. Evolution 57: 1707-1722.
485 Gómez D, Conejeros P, Marshall S, Consuegra S (2010) MHC evolution in three
486 salmonid species: a comparison between class II alpha and beta genes.
487 Immunogenetics 62: 531-542.
488 Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. J
489 Heredity 86: 485-486.
490 Grimholt U, Drablos F, Jorgensen SM, Hoyheim B, Stet RJ (2002) The major
491 histocompatibility class I locus in Atlantic salmon (Salmo salar L.):
492 polymorphism, linkage analysis and protein modelling. Immunogenetics 54: 570
493 - 581.
494 Grimholt U, Hordvik I, Fosse VM, et al. (1993) Molecular cloning of major
495 histocompatibility complex class I cDNAs from Atlantic salmon (Salmo salar).
496 Immunogenetics 37: 469-473.
497 Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis
498 program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95-98.
499 Hammer O, Harper D, Ryan P (2001) PAST: Paleontological statistics software package
500 for education and data analysis. Palaeontol Electron 4: 9.
501 Hedrick P, Kim T (2000) Genetics of complex polymorphisms: parasites and
502 maintenance of MHC variation Harvard University Press, Cambridge, MA.
503 Hendry AP, Farrugia TJ, Kinnison MT (2008) Human influences on rates of phenotypic
504 change in wild animal populations. Mol Ecol 17: 20-29.

505 Huchard E, Weill M, Cowlishaw G, Raymond M, Knapp LA (2008) Polymorphism,
506 haplotype composition, and selection in the Mhc-DRB of wild baboons.
507 Immunogenetics 60: 585-598.
508 Hughes AL, Yeager M (1998) Natural selection at major histocompatibility complex
509 loci of vertebrates. Ann Rev Genet 32: 415-435.
510 Janeway CA, Travers P, Walport D, Shlomchik MJ (2004) Immunobiology: The
511 Immune System in Health and Disease.
512 Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release
513 hypothesis. Trends Ecol Evol 17: 164-170.
514 Kennedy CR, Pojmanska T (1996) Richness and diversity of helminth parasite
515 communities in the common carp and in three more recently introduced carp
516 species. J Fish Biol 48: 89-100.
517 King TL, Eackles MS, Letcher BH (2005) Microsatellite DNA markers for the study of
518 Atlantic salmon (Salmo salar) kinship, population structure, and mixed-fishery
519 analyses. Mol Ecol Notes 5: 130-132.
520 Kurtz J, Kalbe M, Aeschlimann P, et al. (2004) Major histocompatibility complex
521 diversity influences parasite resistance and innate immunity in sticklebacks.
522 Proceedings of the Royal Society of London Series B 271, 197 - 204.
523 Landry C, Garant D, Duchesne P, Bernatchez L (2001) “Good genes as heterozygosity":
524 the major histocompatibility complex and mate choice in Atlantic salmon
525 (Salmo salar). Proc R Soc B-Biol Sci 268: 1279-1285.
526 Lee KA, Klasing KC (2004) A role for immunology in invasion biology. Trends Ecol
527 Evol 19, 523-529.
528 Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA
529 polymorphism data. Bioinformatics 25, 1451.

530 Lowe S, Browne M, Boudjelas S (2000) 100 of the world's worst invasive alien species.
531 In: IUCN/SSC Invasive Species Specialist Group, Auckland, New Zealand.
532 Mays Jr HL, Hill GE (2004) Choosing mates: good genes versus genes that are a good
533 fit. Trends Ecol Evol 19: 554-559.
534 Mitchell CE, Power AG (2003) Release of invasive plants from fungal and viral
535 pathogens. Nature 421, 625-627.
536 Molnar JL, Gamboa RL, Revenga C, Spalding MD (2008) Assessing the global threat
537 of invasive species to marine biodiversity. Front Ecol Environ 6: 485-492.
538 Monzón-Argüello C, Garcia de Leaniz C, Gajardo G, Consuegra S (2013) Less can be
539 more: loss of MHC functional diversity can reflect adaptation to novel
540 conditions during fish invasions. Ecol Evol 3: 3359-3368.
541 Murrell B, Moola S, Mabona A, et al. (2013) FUBAR : A Fast, Unconstrained Bayesian
542 AppRoximation for inferring selection. Mol Biol Evol 30: 1196-1205.
543 Murrell B, Wertheim JO, Moola S, et al. (2012) Detecting Individual Sites Subject to
544 Episodic Diversifying Selection. PLoS Genet 8, e1002764.
545 Naylor RL, Williams SL, Strong DR (2001) Aquaculture--A Gateway for Exotic
546 Species. Science 294: 1655-1656.
547 O'Reilly P, Hamilton L, McConnell S, Wright J (1996) Rapid analysis of genetic
548 variation in Atlantic salmon (Salmo salar) by PCR multiplexing of dinucleotide
549 and tetranucleotide microsatellites. Can J Fish Aq Sci 53: 2292-2298.
550 Olafsson K, Hjorleifsdottir S, Pampoulie C, Hreggvidsson GO, Gudjonsson S (2010)
551 Novel set of multiplex assays (SalPrint15) for efficient analysis of 15
552 microsatellite loci of contemporary samples of the Atlantic salmon (Salmo
553 salar). Mol Ecol Resour 10: 533-537.

554 Olsen KH, Grahn M, Lohm J, Langefors A (1998) MHC and kin discrimination in
555 juvenile Arctic charr, Salvelinus alpinus (L.). An Behav 56: 319-327.
556 Penn D, Potts WK (1998) Chemical signals and parasite-mediated sexual selection.
557 Trends Ecol Evol 13: 391-396.
558 Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic
559 costs associated with alien-invasive species in the United States. Ecol Econ 52:
560 273-288.
561 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using
562 multilocus genotype data. Genetics 155: 945-959.
563 Radwan J, Biedrzycka A, Babik W (2010) Does reduced MHC diversity decrease
564 viability of vertebrate populations? Biol Cons 143: 537-544.
565 Rice WR (1989) Analyzing tables of statistical tests. Evolution 43: 223-225.
566 Roberts SC, Hale ML, Petrie M (2006) Correlations between heterozygosity and
567 measures of genetic similarity: implications for understanding mate choice. J
568 Evol Biol 19: 558-569.
569 Roberts SC, Little AC, Gosling LM, et al. (2005) MHC-assortative facial preferences in
570 humans. Biol Lett 1: 400-403.
571 Sandberg M, Eriksson L, Jonsson J, Sastram M, Wold S (1998) New Chemical
572 Descriptors Relevant for the Design of Biologically Active Peptides. A
573 Multivariate Characterization of 87 Amino Acids. J Med Chem 41: 2481-2491.
574 Schwensow N, Fietz J, Dausmann KH, Sommer S (2007) Neutral versus adaptive
575 genetic variation in parasite resistance: importance of major histocompatibility
576 complex supertypes in a free-ranging primate. Heredity 99: 265-277.

577 Shriner D, Nickle DC, Jensen MA, Mullins JI (2003) Potential impact of recombination
578 on sitewise approaches for detecting positive natural selection. Genet Res 81:
579 115-121.
580 Simberloff D, Martin J-L, Genovesi P, et al. (2013) Impacts of biological invasions:
581 what's what and the way forward. Trends in Ecology and Evolution 28, 58-66.
582 Slade RW, McCallum HI (1992) Overdominant vs. frequency-dependent selection at
583 MHC loci. Genetics 132: 861-864.
584 Slatkin M (1994) An exact test for neutrality based on the Ewens sampling distribution.
585 Genet Res 64:71-74
586 Slatkin M (1996) A correction to the exact test based on the Ewens sampling
587 distribution. Genetical Research 68:259-260
588 Slettan A, Olsaker I, Lie Ø (1995) Atlantic salmon, Salmo salar, microsatellites at the
589 SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. Anim Genet 26: 281-282.
590 Strayer DL, Eviner VT, Jeschke JM, Pace ML (2006) Understanding the long-term
591 effects of species invasions. Trends Ecol Evol 21: 645-651.
592 Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced
593 species and their missing parasites. Nature 421: 628-630.
594 Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and
595 animals. Front Ecol Environ 2: 183-190.
596 Vasemagi A, Nilsson J, Primmer CR (2005) Expressed Sequence Tag-Linked
597 Microsatellites as a Source of Gene-Associated Polymorphisms for Detecting
598 Signatures of Divergent Selection in Atlantic Salmon (Salmo salar L.). Mol Biol
599 Evol 22: 1067-1076.
600 Watterson GA (1978) The homozygosity test of neutrality. Genetics 88:405-417

601 Wayne D, Poon AF, Frost SDW, Pond SLK (2010) Datamonkey 2010: a suite of
602 phylogenetic analysis tools for evolutionary biology. Bioinformatics 26: 2455-
603 2457.
604 Wegner KM, Reusch TBH, Kalbe M (2003) Multiple parasites are driving major
605 histocompatibility complex polymorphism in the wild. J Evol Biol 16: 224-232.
606 Wetzlar HJ (1979) Beiträge zur Biologie und Bewirtschaftung von Forellen (Salmon
607 gairdnerii und S. trutta) in Chile, Albert-Ludwigs-Universität.
608 White TA, Perkins SE (2012) The ecoimmunology of invasive species. Funct Ecol 26:
609 1313-1323.
610 Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, 'invasive traits'
611 and recipient communities: challenges for predicting invasive potential. Divers
612 Distrib 14: 569-580.
613 Yang Z, Swanson W (2002) Codon-substitution models to detect adaptive evolution that
614 account for heterogeneous selective pressures among site classes. Mol Biol Evol
615 19: 49-57.
616 Yang ZH (2007) PAML 4: Phylogenetic analysis by maximum likelihood. Mol Biol
617 Evol 24: 1586 - 1591.
618 Young KA, Dunham JB, Stephenson JF, et al. (2010) A trial of two trouts: comparing
619 the impacts of rainbow and brown trout on a native galaxiid. Anim Conserv 13,
620 399-410.

621