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Winning the invasion roulette: escapes from fish farms increase admixture and facilitate establishment of non-native rainbow trout

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Abstract

Aquaculture is a major source of invasive aquatic species, despite the fact that cultured organisms often have low genetic diversity and tend to be maladapted to survive in the wild. Yet, to what extent aquaculture escapees become established by means of high propagule pressure and multiple origins is not clear. We analysed the genetic diversity of 15 established populations and four farmed stocks of non-native rainbow trout in Chile, a species first introduced for recreational fishing around 1900, but which has in recent decades escaped in large numbers from fish farms and become widespread. Aquaculture propagule pressure was a good predictor of the incidence of farm escapees, which represented 16% of all free-ranging rainbow trout and were present in 80% of the study rivers. Hybrids between farm escapes and established trout were present in all rivers at frequencies ranging between 7 and 69%, and population admixture was positively correlated with genetic diversity. We suggest that non-native salmonids introduced into the Southern Hemisphere could benefit from admixture because local adaptations may not have yet developed, and there may be initially little fitness loss resulting from outbreeding depression.

Introduction

Human-mediated biological invasions often differ from natural colonizations in a critical aspect, namely the genetic diversity of newly founded populations. Natural colonizations usually involve relatively few individuals with a common origin and result in founder effects, characterized by genetic drift and loss of genetic diversity (Nei et al. 1975). In contrast, anthropogenic invasions tend to involve individuals from diverse origins that may harbour relatively high levels of genetic variation (Frankham 2005; Wares et al. 2005).

Given that genetic variability is necessary for populations to respond adaptively to environmental change (Reznick et al. 1997), a diverse origin could augment the genetic variation of newly founded populations and increase their invasive potential (Sexton et al. 2002). When individuals from different sources interbreed, the resulting genetic admixture can create novel genetic combinations, which may facilitate rapid adaptation to novel conditions (Kolbe et al. 2004, 2007). Knowledge on the origin and extent of genetic variation of founder populations, therefore, may be important for understanding establishment success of invasive species.

Freshwater ecosystems exhibit relatively high levels of endemism, and this can exacerbate the impact of non-native fish introductions, which have in many cases originated from accidental aquaculture escapes or the deliberate stocking for recreational fishing (Cambray 2003; Casal 2006; Leprieur et al. 2008). However, aquaculture escapees differ from other invaders in several key traits, which make predictions about their likely impacts challenging. Fish escaping from fish farms tend to be characterized not only by high propagule pressure and high growth rates, traits that may increase establishment success, but also by reduced genetic variability and

behavioural deficits, traits that will tend to curtail dispersal and minimize establishment (Gross 1998; Naylor et al. 2005).

Rainbow trout (*Oncorhynchus mykiss*) is one of the most widespread fish invaders in the world, having been introduced to 90 countries worldwide (Casal 2006) and being listed as one of the '100 World's Worst Invasive Alien Species' (ISSG 2008). In Chile, rainbow trout was introduced deliberately for sport fishing in the early 1900s, although the provenance of early introductions is poorly documented (Basulto 2003). More information is available about the second, more recent wave of salmonid invasions that originated in the late 1980s with the exponential growth of the Chilean salmon industry, the second largest in the world (Gajardo and Laikre 2003). Currently, up to 4 million salmon and trout escape annually from fish farms in Chile (Arismendi et al. 2009) and are now present throughout Chilean Patagonia (Soto et al. 2006; Young et al. 2010).

In their native range, salmonids tend to be locally adapted (Garcia de Leaniz et al. 2007), and this makes them particularly vulnerable to genetic introgression from farm escapees (Rhymer and Smberloff 1996; Boyer et al. 2008), which can breakdown locally coadapted gene complexes and result in low hybrid fitness (McGinnity et al. 2003). Yet the effects of farm escapes on salmonid populations outside their native range are unknown. Such knowledge is important because the invasion success of non-native salmonids escaping from fish farms could be either facilitated or hampered by the presence of established 'naturalized' salmonids originating from previous introductions. Therefore, the question remains whether invasive salmonids escaping from fish farms are able to survive and interbreed with established populations and whether such outbreeding increases genetic diversity and fitness or, on the contrary, breaks down locally adapted gene complexes and reduces competitive ability.

We used microsatellite DNA markers to assess the genetic diversity and likely origin of 15 rainbow trout populations in the Los Lagos Region (Chilean Patagonia) possibly the region with the largest concentration of open-net salmonid farms anywhere in the world (Buschmann et al. 2009). We also analysed four farm populations near the study rivers to investigate the potential scope for interbreeding between escapees and wild individuals. We aimed to test two hypotheses, namely that (i) the diversity non-native rainbow trout in Chile is high as a result of admixture between populations with diverse genetic origins and (ii) that interbreeding between individuals escaping from fish farms and 'naturalized' trout contributes to increase the genetic diversity (and possibly the fitness) of established wild populations.

Material and methods

Sample collection

Free-living rainbow trout ($n = 314$; fork length 40–565 mm) were caught by a combination of single-pass electrofishing (LR-24; Smith-Root Corporation, Vancouver, WA, USA) and angling at fifteen first- to third-order streams (average width 0.8–8.0 m) in the Los Lagos Region during 2007–2009 (Fig. 1A). These were referred to as 'wild' fish and included anadromous as well as resident individuals. We concentrated our sampling in the lower reaches of streams (five of which are described in Young et al. 2010), as these will often represent the main invasion routes for aquaculture escapees. Farmed rainbow trout ($n = 125$; fork length 43–495 mm) were collected at four nearby commercial fish farms (Fig. 1A) during the same period. Twenty free-living individuals from two different rivers had phenotypic traits normally associated with farmed fish, such as short opercula and eroded fins (Thorstad et al. 2008), and were therefore likely recent farm escapees. To assess our ability to identify such escapees, two observers working independently examined 40 photographs of trout and classified them as 'wild' or 'escapees' without prior knowledge of the origin of the fish or the location of capture.

Adipose fin clips from all fish were collected and preserved in 90% ethanol at 4°C for subsequent genetic analyses. Fork length, measured from the tip of the snout to the fork of the tail (FL, mm), and wet weight (W, g) were determined for a subsample of 241 individuals, and Fulton's condition factor ($CF = W \times 10^5/FL^3$) was estimated as a proxy for nutritional status, as this trait correlates well with food intake and growth rate and is known to be under strong selection in the wild (Svanbäck and Persson 2009). As survival of aquaculture escapees depends crucially on their ability to find natural food items after they escape, changes in body condition can therefore reflect the extent to which salmonid escapees forage efficiently in the wild (Schröder and Garcia de Leaniz 2011).

Estimation of salmonid propagule pressure from fish farms

Coordinates of each sampling site were obtained using a GPS (GARMIN Colorado™ Series, Southampton, Hampshire, UK), while coordinates of each registered salmonid farm in the study area (378 off Chiloé Island and 18 in the three study lakes) were obtained from official sources (Department of Aquaculture, SUBPESCA, September 2008), from the Chilean Aquaculture Farm Guide (4th Edition 2001, La Tene Maps, http://www.latene.com/) and from Google Earth. Rainbow trout make up 24% of the smolt production at the study sites, the rest being Atlantic

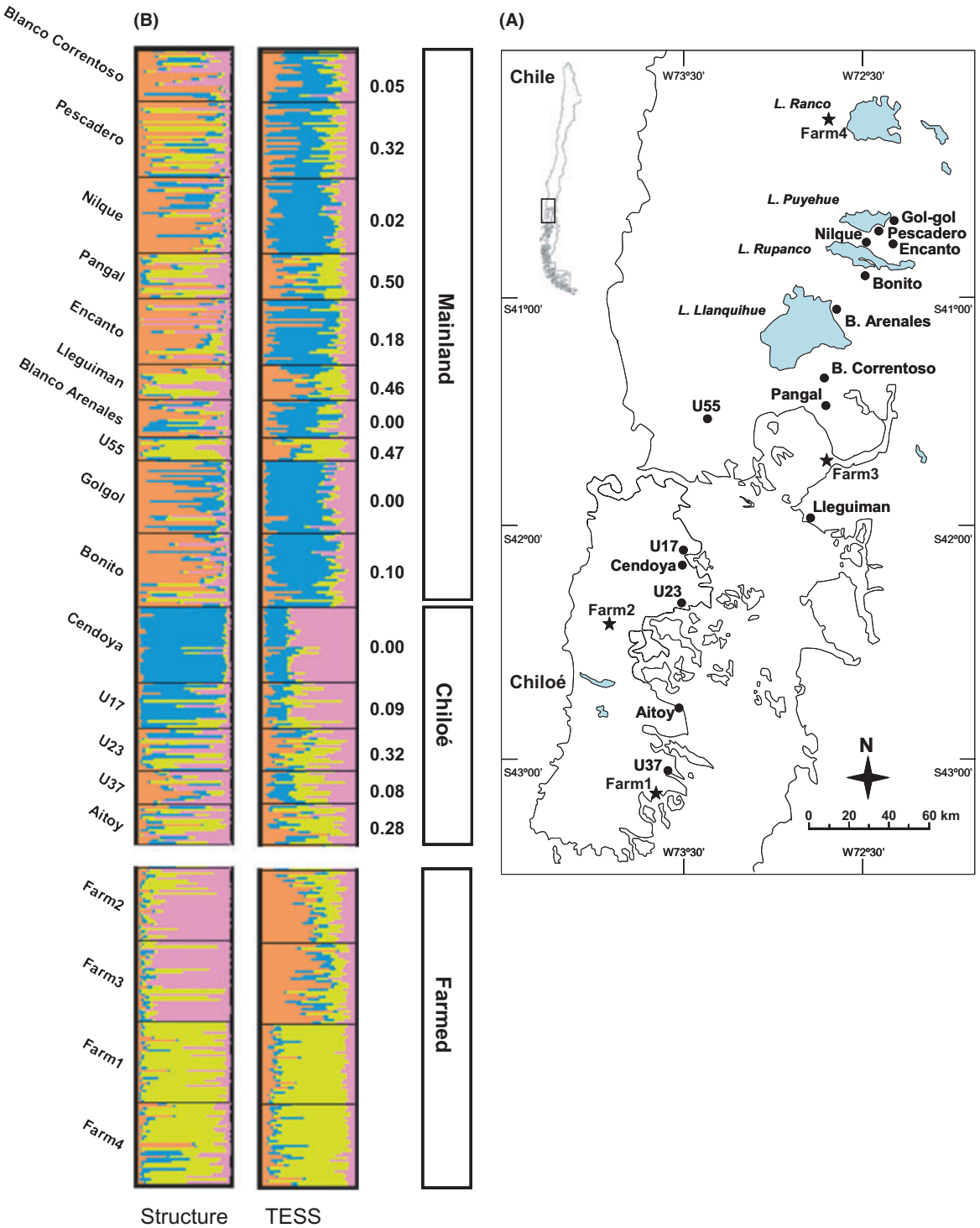

Figure 1 (A) Study rivers (●) and location of rainbow trout farms (★) sampled in Chilean Patagonia; (B) Bayesian clustering of rainbow trout of farmed and wild origin (Chiloé vs. Mainland) according to STRUCTURE and TESS assuming four inferred clusters (K = 4). Each horizontal bar represents an individual fish, with colours representing the probability of membership to each of the clusters (different colours are used by the two programmes). Numbers alongside each river represent the estimated proportion of trout of pure farmed origin, which are likely to be recent escapees.

salmon (50%) and coho salmon (24%; León-Muñoz et al. 2007). Although we did not have data on the species breakdown at all farms, data from 104 farms indicated that most companies (77%) farmed rainbow trout in combination with Atlantic and Pacific salmon at the same sites. Thus, the spatial distribution of salmonid farms can be considered a good proxy for the distribution of rainbow trout farms.

Production data of individual farms are confidential and not readily available but mean farm area (a surrogate for production) was of 5052 m^2 (SE = 630.6) and not different among sites ($F_{2, 15} = 0.368$, $P = 0.686$). We therefore used the weighted distance ($\Sigma 1/d$) from each sampling site to every salmonid farm within a 100-km radius to calculate an index of aquaculture propagule pressure and thus to predict the likely contribution of trout escapees at each study site.

There are no data on the movements of salmonid escapees in Chile, but results from Norway indicate that rainbow trout can travel up to 25–40 km within a week after escaping from fish farms (Skilbrei and Wennevik 2006). Thus, our chosen 100-km radius seems like a reasonable distance to model propagule pressure and included 59% of all the possible pairwise distances to farms located off Chiloé Island and 100% of all the possible distances to farms located at the study lakes (Fig. 1A).

DNA extraction and amplification

Total genomic DNA was extracted using the Wizard® SV 96 Genomic DNA purification kit (Promega, Madison, WI, USA) according to manufacturer's instructions. Two microlitres of extracted DNA (10–15 ng) was used for a single multiplex PCR of 10 microsatellites using the QIAGEN Multiplex PCR kit (QIAGEN, Sussex, UK) in a total volume of 8.5 μL. Forward microsatellite primers were 5′-labelled with one of four dyes as follows: *VIC*-OMM5188, *NED*-OMM1741, *NED*-OMM1590 (Coulibaly et al. 2005); *VIC*-OMM5047 (Coulibaly et al. 2005); *PET*-OMM1097 (Rexroad et al. 2002a), *6-FAM*-OMM1501, *NED*-OMM1008 (Rexroad et al. 2002b); 6-FAM OMM3089 (Johnson et al. 2008); *VIC*-Ssa289, *VIC*-Ssa14 (McConnell et al. 1995). Primer concentrations were 2 μM for OMM3089, OMM1051, Ssa289, OMM5188, OMM5047, OMM1008 and OMM1097 and 4 μM for Ssa14, OMM1590 and OMM1741. Amplification conditions consisted of an initial denaturation step of 15 min at 95°C followed by a touchdown PCR that consisted of eight cycles with a 30-s denaturation step at 94°C, a 90-s annealing step starting at 64°C and descending in 2-cycle steps of 2°C (64, 62, 60 and 58°C) and 90 s of extension at 72°C. Twenty-four additional cycles of PCR were then performed as above but each with an annealing temperature of 56°C followed by a single final cycle of 10 min at 72°C. Microsatellites were resolved on an Applied Biosystems ABI3130xl Genetic Analyser (Applied Biosystems, Sussex, UK), and fragment length was determined using the GeneScan 500–LIZ size standard and scored using GeneMapper v4.0 (Applied Biosystems).

Genetic diversity

Assigned alleles were checked for genotyping errors using MICRO-CHECKER v2.2.3 (Van Oosterhout et al. 2004). Allele number and allelic richness (standardized by sample size) were calculated for all loci within populations using FSTAT v2.9.3 (Goudet 1995). Allele frequencies per population, observed (H_o) and unbiased expected (H_e) heterozygosities were estimated using GENETIX v4.02 (Belkhir et al. 2000). Differences in allelic richness and heterozygosity among populations were assessed for statistical significance using FSTAT. Deviations from Hardy–Weinberg equilibrium were estimated with GENEPOP v3.4 (Raymond and Rousset 1995), and significance values were adjusted by a Bonferroni correction. The occurrence of genotypic linkage disequilibrium for each pair of loci in each population was tested using GENEPOP.

Our assumption of population connectivity, based on our previous study of the range and abundance of rainbow trout in the region (Young et al. 2010), was that spawning runs in neighbouring rivers might be able to interbreed. However, because the exchange of migrants among rivers was not known, we estimated the effective population size (N_e) of each rainbow trout population using two different methods, one that assumes open populations using COLONY (Jones and Wang 2010) and one that assumes closed populations and random mating using LDNe (Waples and Do 2008). We used allele frequencies ≥0.02 to minimize potential bias caused by rare alleles (Waples and Do 2008).

We tested for evidence of recent genetic bottlenecks among non-native rainbow trout populations in Chilean streams using the two methods implemented in BOTTLENECK v.1.2.02 (Piry et al. 1999).

Genetic differentiation and population structuring

Genetic differentiation was measured by calculating pairwise F_{ST} values between populations using FSTAT and by assessing statistical significance following 10 000 permutations. Given that F_{ST} values can underestimate population differentiation when highly polymorphic microsatellites are used, we also calculated D_{est} values based on allele identities (Jost 2008) using SMOGD (Crawford 2010).

Genetic partitioning of populations and individuals was undertaken using two Bayesian approaches: STRUCTURE

v2.3.2 (Pritchard et al. 2000; Falush et al. 2003) and TESS v2.3.1 (Chen et al. 2007). STRUCTURE assigns fish to groups based on genetic data without any prior information about the origin of the samples (Pritchard et al. 2000; Kaeuffer et al. 2007). TESS, on the other hand, incorporates spatial information and provides more accurate assignments when there is moderate connectivity among populations (Chen et al. 2007), as it was expected to be the case in our samples. These methods assess population structure by detecting departures from Hardy–Weinberg and linkage equilibrium, which would result from recent admixture, migration or hybridization. The most likely number of distinct genetic groups was inferred from the greatest rate of change in the likelihood function with respect to K (ΔK) in STRUCTURE (Evanno et al. 2005) and from the value at which the deviance information criterion (DIC) decreased before reaching a plateau in TESS (Spiegelhalter et al. 2002). We also used the assignment method implemented in NEWHYBRIDS (Anderson and Thompson 2002) to assess the extent of admixture between farm and wild fish and also for investigating the origin of hybrids. NEWHYBRIDS provides the posterior probability that an individual belongs to one of the six possible classes that differ in the extent of admixture, in our case farm, wild and hybrids (F_1, F_2 and backcrosses). We employed HYBRIDLAB (Nielsen et al. 2006) to simulate parental and hybrid genotypes and to estimate the power of admixture analyses to identify the origin of hybrids. To this end, we used 32 pure farm fish and 37 pure wild fish (as classified in STRUCTURE by individual membership values of $q > 0.9$) to simulate the genotypes of 100 individuals from each of the parental and hybrid classes, repeated 10 times. Given the importance that threshold q-values have for identification of hybrids when using STRUCTURE (Vähä and Primmer 2006), we used an admixture model with no prior information and $k = 2$ to define the appropriate q for individual assignment with our set of microsatellites.

We employed hierarchical analysis of molecular variance (AMOVA) to partition genetic variance into among-population contributions using ARLEQUIN v3.1 (Excoffier et al. 2005) and estimated the extent of isolation by distance (IBD) based on a matrix of genetic distances (measured as F_{ST}) and geographical distances using IBDWS v.3.16 (Isolation By Distance Web Service) after 3000 iterations. The relative contribution of drift and gene flow across populations was analysed using the likelihood approach implemented in 2MOD (Ciofi et al. 1999). This was run twice to ensure convergence of results.

Because the potential for admixture (the extent of mixing of previously isolated populations, Balding et al. 2007) may depend not only on the number of different lineages that meet but also on their relative abundances (Keller and Taylor 2010), we calculated Pielou's J' evenness index (Pielou 1966) as an indication of the extent of potential admixture of each population, based on the most likely group membership of each individual (q) derived from STRUCTURE. This index will range from 0.0, if the population is dominated by individuals from only one genetic group, to 1.0 if the population consists of equal numbers of individuals from each genetic group. We then analysed the relationship between Pielou's evenness index and (a) population allelic richness and (b) observed heterozygosity to test the prediction that populations with more admixture should also carry more genetic diversity. Statistical analyses were carried out using SYSTAT v. 10 (SYSTAT 2000).

Results

Genetic diversity

Two microsatellites (Ssa289 and Ssa14) showed null alleles in most populations and were removed from subsequent analyses. We found no evidence of allele dropouts or scoring errors because of stutter peaks. Analysis of linkage disequilibrium was significant in only 3 of 339 pairwise comparisons (between loci and populations). Two of the markers (OMM1051 and OMM5188) deviated significantly from Hardy–Weinberg equilibrium (HW) in more than one population, but were retained because their exclusion did not change the conclusions of our analyses (F_{ST}, N_e, population structuring; data not shown).

All significant F_{Is} values were positive and thus indicative of a slight deficiency of heterozygotes (Supporting information Table S1). Allelic richness ranged between 3.8 and 6.8 globally, and it was not different between farm and wild populations ($P = 0.319$; Table 1). Expected and observed heterozygosities ranged between 0.57 and 0.80 (expected, H_e) and between 0.55 and 0.82 (observed, H_o) and were also not significantly different between farm and wild populations (H_e $P = 0.361$, H_o $P = 0.248$; Table 1).

Analyses of multilocus genotype data indicated small effective (N_e) population sizes (under 100 individuals in most cases) and were generally congruent between the two methods, albeit higher estimates were typically obtained by the linkage disequilibrium method than by COLONY (Supporting information Table S2). We found evidence of recent genetic bottlenecks in only one of the small coastal rivers on the Island of Chiloé (River Aitoy, $P = 0.004$), and this could only be inferred from heterozygosity excesses using one of the two BOTTLENECK methods.

Table 1. Characteristics of 15 wild and four farmed populations of rainbow trout in Chilean Patagonia showing sample size (*N*), number of alleles (*k*), allelic richness (AR), expected heterozygosity (H_e) and observed heterozygosity (H_o) at 8 microsatellite loci. Also given is Pielou's evenness index (J') used to quantify the extent of admixture of individuals belonging to four distinct genetic groups inferred from STUCTURE.

Population	Location	Catchment	Latitude	Longitude	*N*	*k*	AR	He	Ho	J'
Wild										
1. U37	Chiloe	Inner Sea	−43.0332	−73.5745	13	5.000	4.570	0.630	0.580	0.764
2. Aitoy	Chiloe	Inner Sea	−42.7572	−73.5653	16	7.710	6.790	0.800	0.770	0.875
3. U23	Chiloe	Inner Sea	−42.3321	−73.5475	17	7.290	6.370	0.770	0.740	0.977
4. Cendoya	Chiloe	Inner Sea	−42.1529	−73.4961	30	4.860	3.790	0.570	0.550	0.004
5. U17	Chiloe	Inner Sea	−42.1152	−73.4845	18	6.430	5.080	0.690	0.680	0.764
6. Lleguiman	Mainland	Inner Sea	−41.9801	−72.7620	14	7.710	6.590	0.780	0.820	0.756
7. U55	Mainland	Inner Sea	−41.5823	−73.3311	9	5.000	5.000	0.720	0.780	0.622
8. Pangal	Mainland	Inner Sea	−41.4800	−72.6603	18	7.000	5.780	0.740	0.730	0.992
9. Bco. Correntoso	Mainland	Inner Sea	−41.3940	−72.6411	20	6.860	5.640	0.730	0.630	0.668
10. Bco. Arenales	Mainland	L. Llanquihue	−41.0486	−72.6742	15	6.710	5.660	0.710	0.630	0.571
11. Bonito	Mainland	L. Rupanco	−40.8900	−72.4500	29	8.430	6.170	0.770	0.680	0.363
12. Nilque	Mainland	L. Rupanco	−40.7840	−72.4347	30	7.140	5.560	0.740	0.630	0.216
13. El Encanto	Mainland	L. Puyehue	−40.7840	−72.3349	26	8.290	6.130	0.760	0.700	0.470
14. Pescadero	Mainland	L. Puyehue	−40.7840	−72.4051	30	8.570	5.960	0.760	0.660	0.817
15. Gol–Gol	Mainland	L. Puyehue	−40.7840	−72.3300	29	7.290	5.290	0.720	0.700	0.216
Farmed										
1. Farm 1	Chiloe	Inner Sea	−43.1164	−73.6323	32	7.710	5.490	0.710	0.650	0.004
2. Farm 2	Chiloe	Inner Sea	−42.4206	−73.9195	29	7.570	6.270	0.800	0.760	0.480
3. Farm 3	Mainland	Inner Sea	−41.7326	−72.6339	32	9.140	6.490	0.780	0.780	0.555
4. Farm 4	Mainland	L. Ranco	−40.1965	−72.6258	32	7.710	5.960	0.770	0.690	0.201

Population structuring

F_{ST} differentiation was not significantly different ($P = 0.806$) between farms ($F_{ST} = 0.064$) and wild populations ($F_{ST} = 0.073$). Global F_{ST} was 0.095. D_{est} values were 0.226 for farm and 0.229 for wild trout and displayed the same trend of pairwise differentiation shown by F_{ST} values (Supporting information Table S3). We did not detect a pattern of isolation by distance, regardless of whether all the populations were included ($Z = -55037.9062$, $r = -0.1653$, one sided $P = 0.8350$) or only the wild trout were considered ($Z = -14055.1449$, $r = 0.0340$ one sided $P = 0.3610$).

AMOVA results indicated that group of origin (farm versus wild) explained 2.3% of genetic variation, while 6.9% of variation was the result of differences among populations within groups, and 90.7% of variation was because of differences among individuals ($F_{ST} = 0.095$, $P < 0.001$). These results indicate that non-native rainbow trout in Chilean Patagonia show some degree of structuring, albeit this appears to be more influenced by differences among populations than by differences between farmed and wild trout. AMOVA analysis of wild populations grouped by broad geographical origin (Island of Chiloé versus Mainland) was very similar and indicated that 2.1% of variation was explained by differences between geographical groups, 6.3% of variation was explained by differences among populations within each group and 91.7% of variation could be accounted for by differences within trout populations ($F_{ST} = 0.073$, $P < 0.001$). All simulations carried out in 2MOD supported the gene flow–drift model, adding support to the conclusion that the study populations were exchanging individuals and were connected by varying levels of gene flow, likely as a result of interbreeding among anadromous spawners.

Results from TESS and STRUCTURE revealed two possible maxima ($K = 4$ and $K = 7$; Supporting information, Fig. S1), but assuming the existence of four distinct homogeneous genetic groups explained the observed population differentiation better than assuming the existence of seven groups. Individual assignments based on $K = 4$ (Fig. 1B) indicated that wild trout had in general a more diverse genetic origin than individuals sampled at farms, which were genetically more uniform.

Hybrid assignment

Assignments of simulated hybrid classes using STRUCTURE correctly identified all the farm and wild parental fish with a minimum q threshold value of 0.85 (wild mean $q = 0.88$, 95% CI = 0.85–0.91; farm mean $q = 0.88$, 95% CI = 0.86–0.92), and this was therefore the threshold value used for hybrid identification in STRUCTURE and

TESS analyses. Using the same $q = 0.85$ threshold, 100% of the simulated F_1 and F_2 hybrids were correctly identified as admixed (F_1 mean $q = 0.53$, 95% CI = 0.47–0.58; F_2 mean $q = 0.53$, 95% CI = 0.47–0.59). However, 17% of the backcrosses had $q > 0.85$ and could not be distinguished from the parental classes. This suggests that our results are likely to underestimate the true number of hybrids in the study populations.

Using the threshold q-value of 0.85 to identify hybrids from purebred trout, results from both STRUCTURE and TESS were coincident in indentifying hybrids in variable proportions (7–85%) in all populations. The lowest frequency of hybrids was identified in the River Cendoya (7%) and in three of the four farms (Farm 2 = 14%, Farm 1 = 16%, Farm 3 = 16%), while the highest proportion of hybrids was detected in two rivers in the Island of Chiloé (U37 85%, River Aitoy 69%), the area most heavily impacted by fish farms and in one of the rivers flowing into Lake Llanquihue (River Blanco Arenales 80%).

Of the fish classified by STRUCTURE as farm, wild or hybrids based on the 0.85 threshold, NEWHYBRIDS correctly classified 84% of the pure farm (average posterior probability = 0.85, SD = 0.15), 95% of the pure wild (average posterior probability = 0.89, SD = 0.11) and 85% of the hybrids (average posterior probability = 0.89, SD = 0.11), indicating a good agreement between different methods.

A geographical pattern was apparent in the distribution of individuals among genetic groups, and this differentiated farmed from wild trout, as well as wild trout in mainland Chile from those in the Island of Chiloé (Fig. 1B). While regional structuring was more evident from TESS (which uses information on the geographical distribution of samples), results from STRUCTURE tend to show a clearer pattern of ancestry, with largely homogeneous genetic stocks shared among different farms. We found a positive relationship between the extent of population admixture and both allelic richness (multiple regression $F_{2,\ 16} = 4.065$, $R^2 = 0.337$, $P = 0.037$; admixture effect $t = 2.595$, $P = 0.020$, fish origin effect $t = -2.127$, $P = 0.049$) and observed heterozygosity (multiple regression $F_{2,\ 16} = 4.163$, $R^2 = 0.342$, $P = 0.035$; admixture effect $t = 2.709$, $P = 0.015$, fish origin effect $t = -2.000$, $P = 0.063$), suggesting that populations made up of individuals of multiple origins tend to harbour higher levels of genetic diversity than populations consisting of more homogeneous individuals (Fig. 2).

Trout of pure farm origin – and thus likely recent escapees – were found scattered in 12 of the 15 rivers (Fig. 1B), ranging in frequency between 0 and 50% (mean incidence among rivers = 19.2%, SE = 4.78), and represented 16% of all free-living trout sampled (average results from STRUCTURE and NEWHYBRIDS). Farmed,

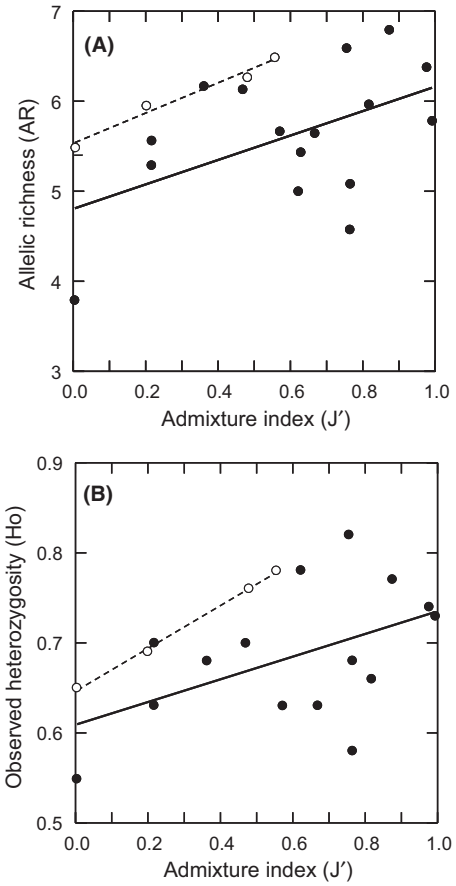

Figure 2 Relationship between genetic admixture estimated from Pielou's evenness index (J′) and genetic diversity measured as (A) allelic richness (AR) and (B) observed heterozygosity (Ho) for farmed (○) and wild rainbow trout populations (●).

recent escapees and wild trout differed greatly in condition factor ($F_{2,\ 237} = 4.380$, $P = 0.014$), as farmed fish had a significantly higher condition factor (mean = 1.43, SE = 0.025) than wild fish (mean = 1.31, SE = 0.024; *Post hoc* Bonferroni adjusted $P = 0.010$), which did not differ from that of escapees (mean = 1.36, SE = 0.047; *Post hoc* Bonferroni adjusted $P = 1.000$).

Agreement in the visual identification of escapees from photographs was 95%. Agreement between phenotypic and genetic assignment of escapees was also high, and 85% of the 20 wild trout classified as likely recent escapees on the basis of their phenotype alone were estimated to have had on average 75% of genetic background of likely farm origin, while only 15% of them were misclassified as belonging to the pure wild group. However, the reverse was not true, and most (82%) of the free-living trout genetically classified as being of pure farm origin had phenotypes that were indistinguishable from those of wild trout.

Aquaculture propagule pressure – measured as the cumulative weighted distance to the fish farms – was a good predictor of the proportion of trout escapees in each study river (Fig. 3; multiple regression $F_{2,\ 12} = 11.376$, $R^2 = 0.655$, $P = 0.002$; propagule pressure effect $t = 4.689$, $P = 0.001$, location effect $t = 4.514$, $P = 0.001$). Differences in intercepts between mainland Chile and Chiloé probably reflect the two different invasion routes in the region: juveniles escaping from hatcheries and smolt farms in lakes and rivers in mainland Chile, and postsmolts escaping from marine net-pens off the Island of Chiloé.

Discussion

Exotic salmonids are now the most abundant freshwater fishes in many parts of the Southern Hemisphere (Soto et al. 2001; McIntosh et al. 2010), where they have caused widespread ecological damage (Garcia de Leaniz et al. 2010), particularly through predation and competition with native fish (McDowall 2003, 2006; Pascual et al. 2007; Young et al. 2009, 2010). As fish invasion success often depends on body size (Schröder et al. 2009) and propagule pressure (Leprieur et al. 2008), the rearing of large number of fast-growing salmonids in open systems in Chilean Patagonia (Soto et al. 2001) may be expected to pose a significant invasion risk.

Our analysis of genetic diversity of Chilean rainbow trout indicates that the contribution of trout escaping from fish farms is substantial, and the incidence of escapees is widespread. Using molecular markers, we estimated

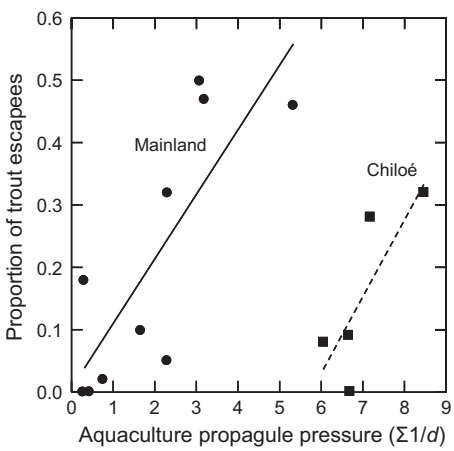

Figure 3 Relationship between propagule pressure (cumulative inversely weighted distance to salmonid fish farms within a 100-km radius) and proportion of rainbow trout escapees in rivers of mainland Chile (●) and the Island of Chiloé (■). Rivers in mainland Chile are mostly affected by escapes from freshwater hatcheries and smolt cages in lakes, while those in Chiloé are mostly affected by escapes from marine net-pens.

that trout escapees were present in 80% of the study streams and represented at least 16% of all free-living rainbow trout sampled, although there was large variation among rivers and our results may have been an underestimate. Variation in the incidence of escapees was well explained by the number and distance to nearby fish farms. Rivers close to fish farms tended to have more trout escapees than rivers located further away, demonstrating the overriding effect of propagule pressure in determining invasion success (Alpert 2006; Reaser et al. 2008; Wilson et al. 2009).

Estimating the degree of admixture becomes especially challenging when gene frequencies are unknown in the original populations prior to admixture (Beaumont et al. 2001). In this sense, and using the simulations performed in HYBRIDLAB, our study parameters (8 microsatellite markers and threshold value of $q = 0.85$) are expected to provide a reasonably accurate estimate of the incidence of hybrids, the main problem being the potential misclassification of backcrosses as purebreds, which may have underestimated the true degree of admixture. The high concordance (84–90%) in the identification of hybrids by three different methods suggests that our estimates of admixture and incidence of trout escaping from fish farms are probably sound. Yet, while fish that were phenotypically identified as recent escapees were in 85% of cases also genetically identified as such, the reverse was not true, and most genetically identified escapees showed no obvious phenotypic differences from wild fish, probably because they had escaped at a young age or had quickly regenerated their fins. This can have important implications for conservation because it suggests that phenotypic identification alone can grossly underestimate the incidence of salmonid escapees in the region.

Our study indicates the existence of at least four ancestral lineages among Chilean rainbow trout, as well as considerable hybridization among lineages, particularly among free-ranging 'wild' trout. A positive relationship was detected between the degree of genetic admixture and genetic diversity, suggesting that the number of source (founding) trout populations has an additive effect on within-population genetic diversity, which may increase fitness and facilitate adaptation (Reed and Frankham 2003; Kolbe et al. 2007) if admixture also enhances selectively important variation.

Phenotypic data provide compelling evidence for the presence of fish of farm origin (escapees) in these rivers, while genetic data provide clear evidence of hybridization of fish from different lineages, at least two of them of farm origin. This strongly suggests that fish escaping from fish farms are not only surviving and entering the streams to spawn, but also significantly contributing to the genetic diversity of the study populations. Moreover, our results

suggest that escapees do not necessarily have low condition factor (used here as a proxy for nutritional status), suggesting that contrary to previous indications (Valiente et al. 2010), farm lineages can perform well in cases when native populations are absent and/or when they are found admixed with other genetic backgrounds. Rainbow trout farming began in the region in the 1980s, but only consistently surpassed 550 tonnes annually since 1999 (Buschmann et al. 2006, Arismendi et al. 2009). Thus, although the species was first introduced to parts of Chile for over a century (Basulto 2003), in reality many rainbow trout populations in Patagonia may be <10 years old (2–3 generations). The recent history of salmonid farming, the high incidence of escapees and F_1–F_2 hybrids and the fact that there are still uninvaded neighbouring streams in the region (Young et al. 2010) would suggest that many of our study populations have only recently been invaded. If so, a recent origin may have prevented the development of locally adapted populations, and therefore, the benefits of genetic admixture may still outweigh the costs of outbreeding (Verhoeven et al. 2011).

We found relatively small effective population sizes (<100) in nearly all cases, but no compelling evidence of recent bottlenecks, apart from one single population and only in one of the analyses. Most populations displayed relatively high levels of genetic diversity, as measured by allelic richness and heterozygosity, which were in any case similar between wild and farmed populations, perhaps owing to the large number and diverse origin of trout farms in the region (León-Muñoz et al. 2007). In any case, it would appear that exotic rainbow trout in Chile are able to maintain genetic diversity despite having small effective population sizes. The only exception to this pattern is the River Cendoya, the population with the smallest N_e, the lowest genetic diversity, but also the lowest degree of admixture and incidence of escapees. Rainbow trout populations in Chilean Patagonia seem to be connected by gene flow, although we did not find evidence of isolation by distance. This could be explained by the relatively short evolutionary time since these populations were founded, but perhaps also by the confounding presence of farm escapees (which would tend to homogenize populations). Gene flow could also help to explain the relatively high level of genetic diversity exhibited by these populations. Indeed, genetic diversity can originate as much from gene flow (Eales et al. 2008), as from admixture among different genetic origins (Kolbe et al. 2008), even among populations founded from a restricted number of sources, as it appears to have been the case in Chile.

Salmonids rank among the most pervasive fish invaders (Fausch 2008; Pascual et al. 2009), despite the farm origin of many of the initial founder stocks (Riva Rossi et al. 2004; Valiente et al. 2010). Salmonid populations of farm origin appear to be less successful invaders than populations of wild origin (Soto et al. 2001; Valiente et al. 2010), although as our study indicates, invasion success of non-native salmonids will also depend strongly on propagule pressure. Trout of farm origin, hence, may achieve high establishment success if propagule pressure is high enough. This could explain why rainbow trout seem to have colonized significantly more streams in Chilean Patagonia than brown trout, despite the fact that both species are facultatively anadromous (Riva-Rossi et al. 2007; Ciancio et al. 2008), appear to spawn at similar times (Estay et al. 2004; Arismendi 2009), have similar abundances, and are found in similar habitats in the region (Young et al. 2010). As brown trout is not commercially farmed, while rainbow trout is, this provides additional – albeit only circumstantial – support for the role of fish farming in facilitating invasions by non-native salmonids in the region. We suggest that rainbow trout is particularly invasive in the region because its spread is aided by escapes from fish farms. Farmed trout may be inherently less fit than wild counterparts, but high propagule pressure and genetic admixture seem to more than compensate for it during the invasion process (Keller and Taylor 2010).

In summary, our results indicate that despite the potential handicap expected from founder effects and loss of genetic diversity during the invasion process (Frankham 2005), multiple origins and admixture resulting from farm escapees seem to have facilitated the invasion of non-native rainbow trout in Chile, allowing the species to spread and to colonize novel environments. As most exotic salmonids are still farmed in open systems in Patagonia and other areas of the Southern Hemisphere (Pascual et al. 2007; Arismendi et al. 2009; Buschmann et al. 2009), better biocontainment, careful zoning and establishment of aquaculture-free areas would appear to be essential tools for managing further spread of farm escapees into these fragile ecosystems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Criteria used for inferring the most likely number of distinct genetic groups within the range $K = 2$–10: (a) changes in Deviance Information Criterion (DIC, mean values ± SD for 20 runs) in TESS and (b) greatest rate of change in the likelihood function with respect to K (ΔK) in STRUCTURE.

Table S1. Values of F_{IS} (SE) for wild and farmed rainbow trout populations in Chilean Patagonia and significance of deviations of genotype frequencies from Hardy Weinberg equilibrium (P). Values in bold are those that remained significant after applying the Bonferroni correction for multiple tests.

Table S2. Estimates of effective population size (N_e) and their 95% confidence intervals for wild and farmed rainbow trout populations using COLONY (Jones and Wang 2010) and the method based on linkage disequilibrium implemented in LDNe (Waples and Do 2008).

Table S3. Pairwise F_{ST} (below diagonal) and D_{est} (above diagonal) values among farmed (F1-F4), and wild populations (BC: Blanco Correntoso, PE: Pescadero, NI: Nilque, PA: Pangal, EE: El Encanto, LL: Lleguiman, BA: Blanco Arenales, U17, U23, U37: Unnamed 13, 23 & 37, AI: Aitoy, U55: Unnamed 55, GG: Golgol, BO: Bonito, CE: Cendoya). F_{ST} values all are significant ($P = 0.001$) based on 10 000 permutations, except those underlined.

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