Genetic characterization of naturalized populations of brown trout *Salmo trutta* L. in southern Chile using allozyme and microsatellite markers

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Abstract

This study describes the genetic structure of five naturalized populations of brown trout in southern Chile using allozyme and microsatellite markers to establish levels of intra- and interpopulation genetic variability and divergence. Fourteen enzymatic systems were used comprising 20 loci and three microsatellite loci specific to brown trout. The genetic variability values (allozymes, $P = 20–35\%$, average = 27%, $H_O = 0.118–0.160$, average = 0.141; microsatellites, $P = 33.3–100\%$, average = 66.66%, $H_O = 0.202–0.274$, average = 0.229) are similar to values described in other naturalized populations of brown trout present in Chile, but higher than those observed in European populations of this species. Values of total genetic diversity (H_T) (allozymes = 0.1216 and microsatellites = 0.3504) and relative genetic divergence (G_{ST}) (allozymes = 9.5% and microsatellites = 15%) were also similar to the results obtained in previous studies of Chilean populations of brown trout. These values, when compared with those obtained in Europe, proved to be similar for H_T but lower for G_{ST}. The low interpopulational genetic differentiation was in accordance with the small genetic distance observed between the populations analysed (D Nei = 0.004–0.025). On the other hand, the high frequency of one of the two alternative alleles of the phylogeographic marker locus *LDH-5** in the populations analysed (*LDH-5*90* > 0.84) would indicate a European origin, in particular Atlantic as opposed to Mediterranean, for the brown trout introduced into Chile. The high levels of genetic variability suggest a mixed origin for the naturalized brown trout in Chile, which could have originated either before or during the introduction process. Nevertheless, the low level of genetic differentiation between populations could reflect the short lapse of time in evolutionary terms, during which populations introduced into Chile have been exposed to different evolutionary forces, and which has not been sufficiently long to produce greater genetic differentiation between populations.

Keywords: brown trout, naturalized populations, allozyme, microsatellite, genetic variability

Introduction

The brown trout, *Salmo trutta* L., has been very successfully introduced around the world from the second half of the nineteenth century onwards (MacCrimmon & Marshall 1968; MacCrimmon, Marshall & Gots 1970). This species, originating from Eurasia and North Africa, was first introduced into Chile at the beginning of the twentieth century with the arrival of stocks imported from Germany (Arratia 1978), although it is believed that subsequent repopulation took place with other stocks of unknown origin (Wetzlar 1979). At present, this species is found in different 'masses' of continental

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waters all along the Chilean coast, ranging from the River Loa in the far north (22°20′S) to Tierra del Fuego in the far south (53°09′S), spanning a latitude of more than 31°.

Numerous studies on the genetic characterization of the brown trout undertaken in its native habitat in central and western Europe, using molecular markers such as mtDNA, allozymes and microsatellites, reveal a high level of intrapopulational genetic variability ($H_o \leq 0.122$) and interpopulational genetic divergence (36.7–56.2%) (Guyomard & Krieg 1983; Ryman 1983; Krieg & Guyomard 1985; Ferguson 1989; Garcia-Marin, Jorde, Ryman, Utter & Pla 1991; Estoup, Presa, Krieg, Vaiman & Guyomard 1993; Presa, Krieg, Estoup & Guyomard 1994; Bertnatchez & Osinov 1995; Riffel, Sorch & Schreiber 1995; Apostolidis, Triantaphyllidis, Kouvatsi & Economidis 1997). This indicates that this species is very substructured, which would concur with the existence of various phylogenetic groups located in more or less precisely defined geographic areas in Eurasia (Bertnatchez, Guyomard & Bonhomme 1992; Bertnatchez & Osinov 1995; Apostolidis *et al.* 1997). High levels of genetic differentiation on a microgeographic scale are also observed in brown trout (Estoup, Rousset, Michalakis, Cornuet, Adriamanga & Guyomard 1998), a situation that has been attributed to the small population sizes and a limited gene flow between populations, partly due to the homing instinct demonstrated by these fishes.

To date, the only published study on the genetic characterization of naturalized brown trout in Chile is that of Faúndez, Blanco, Vázquez & Sánchez (1997), which included an analysis of the genetic structure of seven populations originating from northern, central and southern Chile, using allozymic markers. This study revealed that the mean values of genetic variability in brown trout populations are high, with less interpopulational genetic divergence than in European populations. The high frequency of the allele 90 (>0.7) of the phylogeographic marker locus *LDH-5** (Hamilton, Ferguson, Taggart, Tomasson, Walker & Fahy 1989) suggested a European, in particular Atlantic ('modern race'), as opposed to Mediterranean ('ancestral race'), origin for the brown trout in Chile.

The brown trout is a valuable resource in our country, especially in recreational sport fishing. This is an important activity in the south of Chile where this salmonid inhabits numerous rivers and lakes. Nevertheless, over the last few decades, populations of this species have decreased in this area as a consequence of excessive fishing and chemical and/or biological pollution of the waters (D. Soto, pers. comm.). For this reason, a restocking programme must be initiated to restore these populations. Information on the reproductive cycle and genetic structure is indispensable to the development of effective conservation and/or exploitation plans for this species in Chile.

This study reports on the genetic characterization of five naturalized populations of brown trout in southern Chile (regions IX and X) using allozyme and microsatellite markers, with the objective of contributing to existing information and thus ensuring the conservation and rational exploitation of this species in our country. Similarly, our research broadens the scope of the study previously undertaken by Faúndez *et al.* (1997), as only one population from this geographic region was studied on that occasion.

Materials and methods

The five populations analysed were located in the following rivers: Pichil (40°42′S 72°58′W, region X), Claro (39°17′S 71°56′W, region IX), Zahuil (39°35′S 72°13′W, region X), Huilma (40°43′S 73°13′W, region X) and Chanchán (40°45′S 73°00W, region X). The Pichil, Huilma and Chanchán rivers belong to the 'subbasin' of the River Rahue (province of Osorno), which forms part of the hydrographic basin of the River Bueno. The River Claro is a tributary of Lake Villarrica (province of Cautín) and forms part of the hydrographic basin of the River Toltén, whereas the River Zahuil is a tributary of Lake Panguipulli (province of Valdivia), which forms part of the hydrographic basin of the River Valdivia (Fig. 1).

The specimens were collected by electrofishing or hook and line fishing, then transported live to the laboratory for subsequent processing and stored at –80 °C until used. Samples of skeletal muscle, liver and eye were homogenized 1:1 in 0.05 M Tris-HCl buffer, pH 8, centrifuged (2000–4000 *g*) and the supernatant used for horizontal starch electrophoresis at 12.5%. In some cases, the homogenized samples were used without being centrifuged. The enzyme nomenclature for the designation of the loci and alleles follows that of Shaklee, Allendorf, Morizot & Whitt (1990).

Fourteen enzymatic systems representing 20 loci were analysed: malate dehydrogenase (EC 1.1.1.37) (*MDH-1,2**), esterase (EC 3.1.1.-) (*EST-1**, *-2**), isocitrate dehydrogenase (EC 1.1.1.42) (*IDHP-3**),

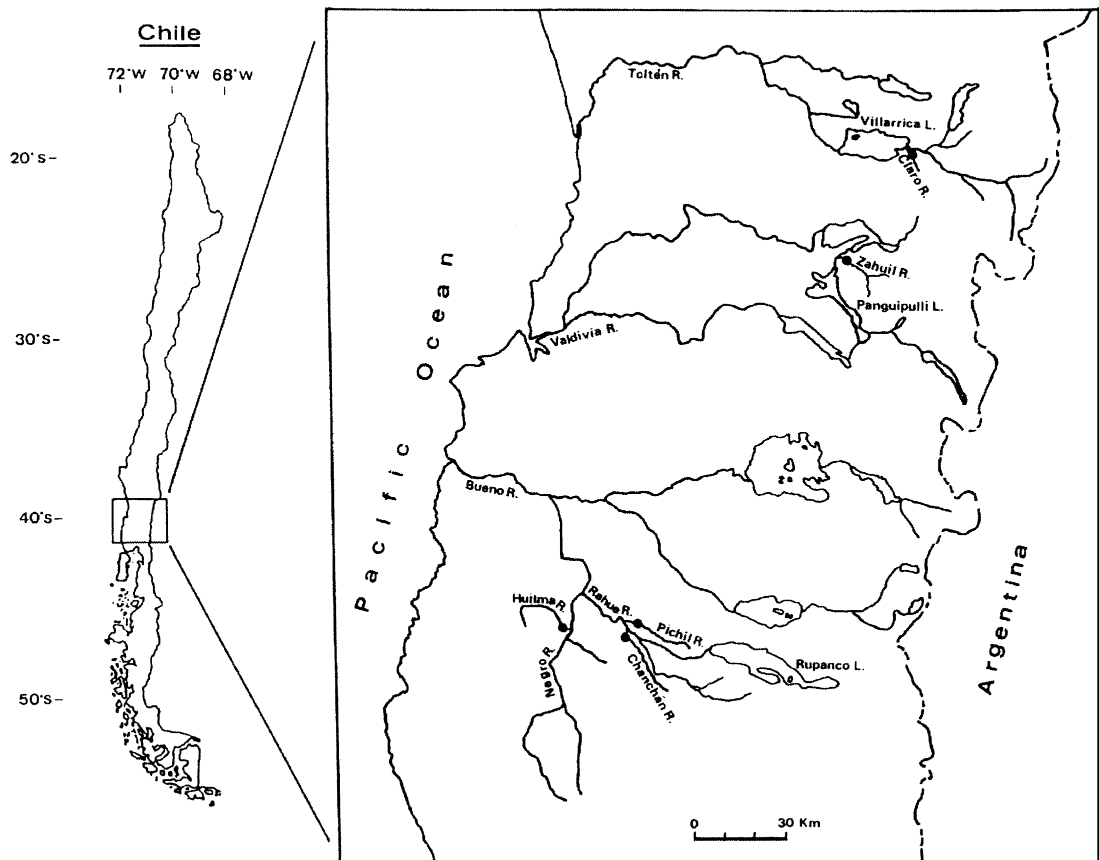

Figure 1 Location in southern Chile of naturalized populations of brown trout sampled in this study. Symbol (filled circles) represents the sampling sites of each population.

phosphogluconate dehydrogenase (EC 1.1.1.44) (*PGDH-2**), glucose-6-phosphate isomerase (EC 5.3.1.9) (*GPI-1**, *-2**, *-3**), aspartate amino transferase (EC 2.6.1.1) (*AAT-2**), L-lactate dehydrogenase (EC 1.1.1.27) (*LDH-5**), phosphoglucomutase (EC 5.4.2.2) (*PGM-2**), superoxide dismutase (EC 1.15.1.1) (*SOD**), alcohol dehydrogenase (EC 1.1.1.1) (*ADH**), glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) (*G3PDH-2**), creatine kinase (EC 2.7.3.2) (*CK-1*, *-2**), malic enzyme-NADP$^+$ (EC 1.1.1.40) (*MEP-1**, *2**) and xanthine dehydrogenase (EC 1.2.3.2) (*XDH**). The buffers used were: Tris–EDTA–citrate, pH 7.1, for the PGDH and EST enzymes; Tris-citrate, pH 8.0, for the AAT, GPI and PGM enzymes; citric acid–EDTA adjusted with N-(3-aminopropyl)-morpholine, pH 6.6, for MDH, IDHP, ADH and SOD; Tris adjusted with NaH_2PO_4, pH 7.4, for LDH, G3PDH, CK and MEP; and Tris–EDTA–borate, pH 9.1, for XDH. Staining was undertaken according to the methods of Shaw & Prassad (1970), Harris & Hopkinson (1976) and Aebersold, Winans, Teel, Milner & Utter (1987).

The analysis of microsatellites involved three populations in the Rivers Pichil, Claro and Zahuil, and considered three specific loci of brown trout: *μStr60**, *μStr15** and *μStr2** (Estoup *et al.* 1993). Blood samples were collected in TNES-urea, after which DNA was obtained from the erythrocytes though digestion with proteinase K, followed by extraction with phenol–chloroform and ethanol precipitation. The microsatellites were amplified by polymerase chain reaction (PCR), using a suitable annealing temperature. The PCR mix (15 μL) contained: 1 × *Taq* polymerase buffer, 100 μM each dNTPs, 1.5 mM $MgCl_2$, 0.2 μM each primer, 0.02 U of *Taq* polymerase (Gibco BRL) and 20 ng of template DNA. The amplification profile was 94 °C (2 min), annealing temperature 48–58 °C (1 min), 72 °C (1 min) followed by 34 cycles and one final cycle at 72 °C (5 min) in an MJ Research PTC-100 96V programmable thermal cycler. The products of amplification were submitted to electrophoresis using high-resolution agarose Metaphor (FMC) at 3%. A DNA ladder of 100 bp (Gibco BRL) was used as

a size marker, and the alleles were revealed with 0.5 mg mL^{-1} ethidium bromide, using photographs obtained with a Polaroid camera.

Analysis of the data was undertaken using BIOSYS-1 software, version 1.7 (Swofford & Selander 1989). The chi-square test was performed to test for conformance of data to the Hardy–Weinberg model. The Levene (1949) correction factor for small sample size used in chi-square analyses was considered. Heterogeneity of allelic frequencies between the different populations was analysed using the chi-square homogeneity test (Sokal & Rohlf 1981). Genetic diversity (Nei 1973) was used to partition the total amount of genetic variation into its components within and between populations. The unbiased genetic distance was calculated according to Nei (1978) and was subject to unweighted pair group method cluster analysis (UPGMA) (Sneath & Sokal 1973).

Results

Analysis of the allozymic variability revealed that only eight of the 20 loci studied were polymorphics (*MDH-2**, *EST-1**, *EST-2**, *AAT-2**, *G3PDH-2**, *PGM-2**, *CK-1** and *LDH-5**) (Table 1). Polymorphism ($P_{0.05}$ criterion) in the five populations fluctuated between 20% (Rivers Zahuil and Chanchán) and 35% (River Claro), with an average value of 27%. Average observed heterozygosity (H_O) varied between 0.118 (River Chanchán) and 0.160 (River Huilma), with an average value of 0.141 (Table 1). Genetic variability obtained using microsatellites was greater than that observed with allozymes (Table 1). For example, in the three populations analysed, polymorphism fluctuated between 33.3% (River Zahuil) and 100% (River Claro) (average value 66.66%), with H_O values between 0.202 and 0.274 (average 0.229). The probable size of the microsatellite alleles would correspond to 94, 98 and 102 bp for the *μStr60**; 220, 230 and 240 bp for the *μStr15**, and 350 and 352 bp for the *μStr2**.

Statistically significant deviations from the Hardy–Weinberg proportions were observed in 18 of the 28 tests carried out. Two populations (rivers Pichil and Claro) accounted for 11 of these deviations; in both populations, a heterozygous deficiency at *MDH-2**, *G3PDH-2** and *CK-1** loci and an excess at *AAT-2**, *EST-2** and *LDH-5** loci were found.

High statistically significant heterogeneity was found between the five populations in comparisons of the allelic frequencies of both the eight allozymatic loci ($\chi^2 = 239.924$, $P < 0.001$) and the three microsatellite loci ($\chi^2 = 101.923$, $P < 0.001$) (Table 2).

On the other hand, the total genetic diversity (H_T) based on allozymes for the five populations was estimated at 0.1216 (Table 2); 90.5% of this genetic diversity was found within populations (H_M) and only 9.5% between populations (G_{ST}). However, the total genetic diversity, calculated using the microsatellites, produced much higher values than those estimated with allozymes ($H_T = 0.3504$, Table 2). Nevertheless, the relative genetic diversity values between populations were similar.

The genetic distance calculated on the basis of the 20 allozymic loci studied was between 0.004 (River Huilma with River Chanchán) and 0.025 (River Chanchán with River Zahuil), with an average distance of 0.013 (Table 3). The UPGMA dendrogram constructed using these matrix values (Fig. 2) shows that the five populations can be separated into three groups: (1) River Zahuil; (2) Rivers Huilma and Chanchán; and (3) Rivers Pichil and Claro. The River Zahuil is clearly separated from groups 2 and 3.

Discussion

The allozymic variability observed in the five populations of brown trout analysed (Table 1) coincides with the results obtained by Faúndez *et al.* (1997), given that the values they reported are similar to ours (P = 24–36%, average = 29.14% vs. P = 20–35%, average = 27%; H_O = 0.072–0.150, average = 0.110 vs. H_O = 0.118–0.160, average = 0.141). Nevertheless, our results on genetic variability using microsatellites (Table 1) are slightly lower than those reported by Faúndez (1997) when the same type of markers are considered (P = 50–100%, H_O = 0.337–0.650), although he used an additional microsatellite locus (*μStr543**) with greater variation. Thus, our results corroborate previous studies in which a high level of genetic variability was reported for the naturalized brown trout in Chile. This level of polymorphism is in the upper limit of the range described for European populations, in terms of both percentage of polymorphic loci and observed heterozygosity, and has a higher average (P = 0–38%, average = 16%; H_O = 0.017–0.122, average = 0.044) (Krieg & Guyomard 1985; Ferguson 1989; Apostolidis, Karakousis & Triantaphyllidis 1996). In the case of microsatellite variability, our results are similar to those obtained by Presa *et al.* (1994).

Table 1 Observed allelic frequencies of polymorphic loci and estimates of average observed ($H_O \pm$ EE) and expected ($H_E \pm$ EE) heterozygosities and proportion of polymorphic loci (*P* 0.05 criterion) for allozyme and microsatellite markers in naturalized populations of brown trout from five localities in southern Chile

	Populations				
Loci/alleles	Pichil	Claro	Zahuil	Huilma	Chanchán
Allozymes†					
*MDH-2** (*n*)	25	24	26	18	26
100	0.520	0.667	1.000	0.500	0.673
115	0.480	0.333	0.000	0.500	0.327
*EST-1** (*n*)	23	23	26	18	24
100	1.000	0.935	1.000	1.000	1.000
95	0.000	0.065	0.000	0.000	0.000
*EST-2** (*n*)	25	24	26	18	24
100	0.680	0.521	0.654	0.333	0.229
95	0.320	0.479	0.346	0.667	0.771
*AAT-2** (*n*)	25	24	26	13	26
100	0.820	0.938	0.720	0.846	0.962
120	0.100	0.021	0.250	0.154	0.038
110	0.000	0.021	0.019	0.000	0.000
95	0.080	0.021	0.019	0.000	0.000
*G3PDH-2** (*n*)	23	24	25	18	26
100	0.435	0.479	0.260	0.361	0.462
120	0.370	0.479	0.140	0.361	0.462
110	0.065	0.021	0.100	0.222	0.038
90	0.065	0.021	0.280	0.056	0.038
80	0.065	0.000	0.220	0.000	0.000
*PGM-2** (*n*)	23	24	26	18	24
100	1.000	1.000	1.000	0.917	1.000
98	0.000	0.000	0.000	0.083	0.000
*CK-1** (*n*)	23	24	20	18	24
125	0.761	0.521	0.775	0.722	0.917
100	0.239	0.479	0.225	0.278	0.083
*LDH-5** (*n*)	25	18	16	16	26
90	0.840	0.889	1.000	1.000	1.000
100	0.160	0.111	0.000	0.000	0.000
H_O	0.144 ± 0.069	0.157 ± 0.072	0.130 ± 0.062	0.160 ± 0.067	0.118 ± 0.061
H_E	0.130 ± 0.048	0.124 ± 0.046	0.102 ± 0.050	0.126 ± 0.049	0.081 ± 0.039
P 0.05 (%)	30	35	20	30	20
Microsatellites					
*μStr60** (*n*)	25	27	30	–	–
98	0.520	0.815	1.000	–	–
94	0.240	0.093	0.000	–	–
102	0.240	0.093	0.000	–	–
*μStr15** (*n*)	24	26	28	–	–
220	0.479	0.885	0.482	–	–
230	0.458	0.000	0.214	–	–
240	0.063	0.115	0.304	–	–
*μStr2** (*n*)	24	27	30	–	–
350	1.000	0.796	0.983	–	–
352	0.000	0.204	0.017	–	–
H_O	0.202 ± 0.144	0.274 ± 0.068	0.213 ± 0.197	–	–
H_E	0.398 ± 0.200	0.288 ± 0.040	0.225 ± 0.208	–	–
P 0.05 (%)	66.7	100	33.3	–	–

†Average values for allozymes (bottom line) are based on 20 loci.

Table 2 Test of heterogeneity for allelic frequencies and genetic diversity analysis of five naturalized populations of brown trout from southern Chile

	Genetic diversity analysis					
	Test of heterogeneity		*Total diversity*†		Relative diversity‡	
Locus	χ^2	d.f.	H_T	H_S	H_M	G_{ST}
Allozymes						
*MDH-2**	36.032***	4	0.4408	0.3769	85.5	14.5
*EST-1**	12.028*	4	0.0257	0.0243	94.7	5.3
*EST-2**	29.555***	4	0.4994	0.4370	87.5	12.5
*AAT-2**	28.351**	12	0.2550	0.2392	93.8	6.2
*PGM-2**	16.380**	4	0.0327	0.0305	93.2	6.8
*G3PDH-2**	79.361***	16	0.6896	0.6468	93.8	6.2
*CK-1**	20.112***	4	0.3856	0.3532	91.6	8.4
*LDH-5**	18.104**	4	0.1025	0.0932	90.9	9.1
Total	239.924***	52	–	–	–	–
Average§	–	–	0.1216	0.1101	90.5	9.5
Microsatellites						
*μSrt60**	38.476***	4	0.3697	0.3109	84.1	15.9
*μStr15**	43.494***	4	0.5453	0.4635	85	15
*μStr2**	19.953***	2	0.1361	0.1191	87.5	12.5
Total	101.923***	10	–	–	–	–
Average	–	–	0.3504	0.2978	85	15

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. d.f., degrees of freedom.
†H_T, total diversity; H_S, absolute diversity of populations.
‡H_M, relative diversity within populations; G_{ST}, relative diversity between populations.
§H_T and H_S averages were estimated on the 20 loci studied.

Table 3 Genetic distances (below diagonal) and genetic identities (above diagonal) for allozymes in five naturalized populations of brown trout from southern Chile

Populations	1	2	3	4	5
1. Pichil	–	0.995	0.983	0.993	0.986
2. Claro	0.005	–	0.980	0.994	0.988
3. Zahuil	0.017	0.020	–	0.977	0.975
4. Huilma	0.007	0.006	0.023	–	0.996
5. Chanchán	0.014	0.012	0.025	0.004	–

Genetic distances were calculated on the 20 loci analysed.

The brown trout was first introduced into Chile at the beginning of the twentieth century with stocks from Germany; subsequently, other stocks were introduced, but their origin is unknown. The genetic variability observed in this study supports the hypothesis that the origin of the populations of brown trout present in Chile today is mixed as opposed to a single origin resulting from the introduction of one stock from Europe. Although historical antecedents indicate that this 'mixing' process may have occurred during the introduction process (Wetzlar 1979), the possibility that stocks introduced into Chile may already have possessed some degree of 'mix' cannot be discarded. It is interesting to note that hatchery stocks of brown trout used for stocking in some European countries also exhibit a high degree of polymorphism, a fact that has been related to the possible mixed origin of these populations (Garcia-Marin *et al.* 1991). Further studies using mtDNA will permit a more accurate evaluation of the extent to which naturalized brown trout in Chile are of mixed origin.

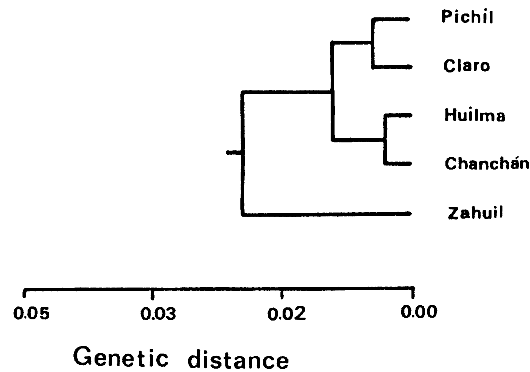

Figure 2 Dendrogram for five naturalized populations of brown trout derived from genetic distances values.

The significant heterogeneity of the allelic frequencies between the populations of brown trout analysed both in allozymes and in microsatellites can be interpreted in a number of ways: a lack of gene flow between populations as a result of geographic isolation; local genetic adaptations to different environmental conditions or partial preservation of the original genetic characteristics of the different stocks that were introduced into the country. Similarly, the occurrence of genetic changes in the original stocks as a result of the low number of trout introduced (founder effect), which provokes genetic drift, causing genetic differentiation in populations, is another possibility that must be contemplated. In this respect, it is probable that, in some cases, geographic isolation may have played an important role in the origin of this genetic heterogeneity, given that some of the populations analysed belong to different hydrographic basins (see Fig. 1), and their isolation occurred from the moment the brown trout were introduced into these basins at the beginning of the twentieth century. Even the homing instinct may be influencing this interpopulational differentiation, given that, in brown trout, this constitutes an important factor in differentiation at the microgeographic level (Estoup *et al.* 1998).

The genetic diversity observed in the present study is high ($H_T = 0.1216$ allozymes and $H_T = 0.3504$ microsatellites; Table 2) compared with values reported for other salmonids (Ryman 1983); only a small percentage of this variation was caused by interpopulational genetic differentiation (allozymes $G_{ST} = 9.5\%$; microsatellites $G_{ST} = 15\%$; Table 2). This result, which coincides with observations made by Faúndez *et al.* (1997), indicates that the genetic divergence between naturalized populations of brown trout in southern Chile is low and, consequently, the highest proportion of genetic variation reported is distributed within the populations. This result is also in accordance with the low genetic distance values registered (range = 0.004–0.025; Table 3), which contrast with descriptions of European populations, where the interpopulational genetic distance and genetic divergence can reach values of up to 0.212 and 0.562 respectively (Apostolidis *et al.* 1996). The low level of interpopulational genetic divergence observed in the present study is in accordance with the results obtained in naturalized populations introduced at a similar period of time in other parts of the world (Krueger & May 1987). This low genetic divergence may reflect the short lapse of time, in evolutionary terms, during which the populations introduced into Chile have been subject to different evolutionary forces, which has not been long enough to produce a greater differentiation between populations.

Various studies carried out in Europe have commented on the presence or absence of certain alleles in the native populations of brown trout, especially in two large geographic regions of that continent: central and north zone (Atlantic region) and southern and Mediterranean zone (Mediterranean region). For example, in the Atlantic region, various alleles can be found that are native to that zone, such as *CK-1*115*, *LDH-3*160* and *LDH-5*90* (Ferguson 1989; Hamilton *et al.* 1989; Apostolidis *et al.* 1996). The last of these three alleles that characterize the 'modern race' of this species in Europe, in contrast to the 'ancestral race', which preferentially exhibits the alternative allele *LDH-5*100* (Hamilton *et al.* 1989), has frequently been used in various studies because of its considerable diagnostic value (Arias, Sánchez & Martínez 1995). Therefore, our results, which reveal a high frequency of the allele *LDH-5*90* (>0.84), as opposed to the allele *LDH-5*100* (Table 1), support the Atlantic origin of the populations of brown trout introduced into Chile. Nevertheless, given that the *LDH-5*100* allele is also present even though at low frequencies in two-fifths of the populations analysed in this study and in five-sevenths of the populations studied by Faúndez *et al.* (1997), it is reasonable to suggest that a certain degree of 'mixing' of stocks occurred between the ancestral and modern groups of the populations, which determined the origin of the brown trout introduced into Chile. Nevertheless, it should be noted that this is a controversial hypothesis as data exist recording the presence of both alleles of *LDH-5** in the native populations of brown trout present in

Germany (Riffel *et al.* 1995), which is where most of the stocks of brown trout introduced into this country would have originated from. It is probable that mtDNA studies in the future will be useful for confirming the type of brown trout stock present in Chile, given that the different evolutionary lineages of this species possess characteristic haplotypes (Bertnatchez *et al.* 1992; Bertnatchez & Osinov 1995).

In this study, we used allozymes and microsatellites to study the genetic structure of the populations. In accordance with the results of studies of other salmonids, where both types of markers were used (Atlantic salmon, Sánchez, Clabby, Ramos, Blanco, Flavin, Vázquez & Powell 1996; brown trout, Faúndez 1997; Estoup *et al.* 1998), our results support the conclusion that the microsatellites are a much more sensitive means of describing genetic variability than allozymes. This result can be explained by the fact that the mutation rate of microsatellites is much higher – between two and four orders of magnitude greater – than that of the allozymes (Estoup *et al.* 1998). Although analyses of genetic diversity demonstrate that the total genetic diversity values obtained with microsatellites were more than double those obtained with allozymes, in terms of the G_{ST} values, these were similar for both types of markers. This similarity in the G_{ST} values is difficult to explain, given that it is presumed that the microsatellites should be more neutral than the allozymes considering their particular location in the non-coding sequences of the genome. In accordance with observations made by Estoup *et al.* (1998), the similarity in the genetic divergence values between populations suggests that selection would not be operating to any great extent in the differentiation of both types of markers.

The brown trout was introduced successfully into Chile approximately one century ago and today forms an integral part of the ichthyofauna of the country (Vila, Fuentes & Contreras 1999). It is probable that, during this period, as a result of adaptation to the local aquatic conditions and/or the founder effect, this species has experienced a certain degree of divergence with respect to the original populations as has been described in naturalized populations in other parts of the world (Krueger & May 1987) and, as a result, its gene pool may possess characteristics that are unique in the world. This study provides genetic information that may be useful when developing plans to ensure the appropriate genetic management of these populations, especially those populations in the southern part of the country. It is imperative that any measures taken contemplate the loss of genetic variability that occurs when new stocks are introduced, as a result of the displacement of existing populations and/or the processes of introgression that may take place (Martínez, Arias, Castro & Sánchez 1993). Thus, the importance of a correct understanding of the genetic structure of naturalized populations must be emphasized before embarking on the formulation of conservation plans for this resource in Chile.

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