Naturalized Chinook salmon in the northern Chilean Patagonia: Do they originate from salmon farming?

Salmones Chinook asilvestrados en el norte de la Patagonia chilena: ¿se originan desde escapes de cultivo?

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Resumen.- En los ríos del sur de Chile se han registrado individuos desovantes de salmón Chinook (*Oncorhynchus tschawytscha*). Esta especie fue introducida a partir del 1900 hasta los 80' y se cultiva actualmente en el mar. Nuestro objetivo fue identificar la procedencia de los salmones Chinook asilvestrados en el río Petrohué, mediante el uso de 3 loci microsatélites. Los resultados fueron comparados con muestras de individuos obtenidos desde cultivo. Los resultados mostraron mayor variabilidad genética en los individuos asilvestrados que la obtenida en los individuos de cultivo y además se observó divergencia genética entre ellos, lo que permite indicar que las poblaciones de salmones desovantes en el río Petrohué actualmente no son explicadas por posibles escapes desde los grupos de salmones de cultivo, sino más bien corresponderían a poblaciones generadas desde los grupos liberados con fines de repoblamiento antes del comienzo de su cultivo.

Palabras clave: Asilvestrados, naturalizados, Chile, *Oncorhynchus tschawytscha*, cultivo

Introduction

Salmonids have been introduced into the rivers and lakes of southern South America since the 1900's, principally for recreational fishing purposes (Golusda 1907, Basulto 2003, Pascual & Ciancio 2007). Introduction of Chinook salmon (*Oncorhynchus tschawytscha* Walbaum) to southern Chile (between 38.7°S and 54.7°S) occurred in five stocking events during the 1970's with the planting of both eggs and alevins from Washington State, U.S.A. (Joyner 1980, Dufflocq 1981, Donaldson & Joyner 1983, Basulto 2003, Pascual & Ciancio 2007), but these initial introductions were described as failures (Basulto 2003). More recently, since the 1980's, there has been a development in intense salmon farming between 40.7°S and 53°S, with Chinook salmon constituting less than 5% of total Chilean farmed fish production (SERNAPESCA 2006). At present, mature salmon have been detected, in Chile, returning to the rivers Pratt (Basulto 2003), Futaleufu (Di Prinzio 2001), and Petrohué (Soto *et al*. 2006, 2007), and in the Argentinean Patagonia (Di Prinzio 2001, Pascual & Ciancio 2007, Becker *et al*. 2007). The Petrohué River is of great relevance because it is the only site with documented Chinook salmon spawning sites and juveniles (Soto *et al*. 2006, 2007). However, it is not clear whether these returning individuals have escaped from farming net pens or are descendents of the first individuals introduced for ranching in the 1970's. Thus, this study genetically compares the spawning Chinook salmon populations in Petrohué River with those from known salmon farming sites to determine whether or not they originated from escaped individuals.

Material and methods

Obtaining the samples

Naturalized specimens were collected from March to April 2006 in the Laguna de Los Patos sector of the Petrohué River basin (41°S, 79ºW) (Fig. 1). Three campaigns were carried out in which 20 spawning Chinook carcasses were captured using backpack electrofishing. Samples were taken from a total of 22 individuals from a farming site on Río Negro-Hornopirén (41.4°S, 72.5°W) in May 2006. This is the only company which raises Chinook salmon in Chile and they have been cultivating this species since 1985 with the same strain of fish. Samples of scales, fins, and muscles of each individual were taken and stored in 1.5-mL tubes with 95% ethanol.

Genetic analysis

DNA was extracted from the fin and scale samples using Proteinase K-Chelex at 10% (Estoup *et al*. 1996). Muscle samples were extracted using the standard method for

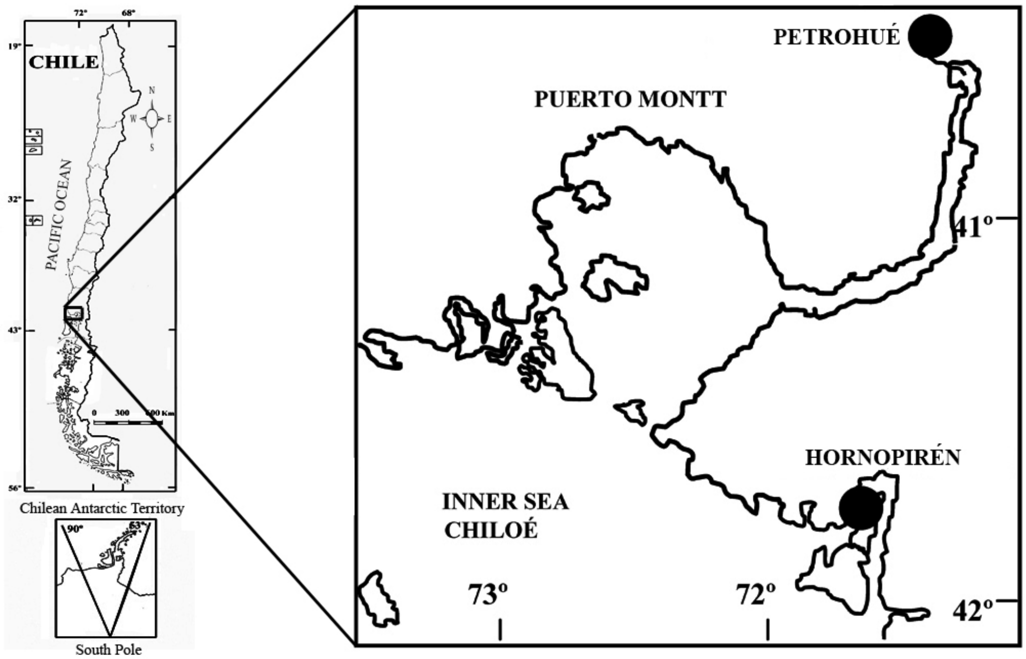

Figure 1

Map of the sampling sites for naturalized (Petrohué) (41°S, 79ºW) and farmed (Hornopirén) (41.4°S, 72.5°W) individuals of Chinook salmon *O. tschawytscha*

Mapa de las localidades de muestreo de individuos asilvestrados (Petrohué) (41°S, 79ºW) y de cultivo (Hornopirén) (41,4°S, 72,5°W) de salmón Chinook *O. tschawytscha*

DNA extraction with phenol-chloroform-isoamylic alcohol followed by precipitation in ethanol. Genotypification was performed on three microsatellites (*Ots*-9, *Ots*-10, *Oneμ*-13) under the conditions described in Greig & Banks (1999). These were then read as a multiplex in an automatic sequencer (ABIPrism 3100).

Statistical analysis

Genetic variability indicators were estimated for each group of individuals, namely observed and expected heterozygosity, average number of alleles per locus, and allelic frequency. The fit to the Hardy-Weinberg equilibrium model was evaluated with the Fisher's exact test using the program GENEPOP v3.1 (Raymond & Rousset 1995). Genotypic disequilibrium values were estimated for comparison of mixed origins between samples. In order to establish the probability that each individual would be assigned to a population, an assignment test was performed using the program GeneClass (Cournet *et al*. 1999). The presence of some population bottleneck process was estimated with the Wilcoxon sign rank test using the program Bottleneck v1.2.02 (Piry *et al*. 1999). The genetic differentiation of the population was evaluated using allelic distribution at different loci through a Fisher's exact test and with F_{st} values for each locus.

Results

On average, the 3 loci analyzed in Chinook salmon had 5.3 alleles per locus (Table 1). This value was higher for the naturalized spawning specimens (N = 4.6, Median = 5) than for the farmed juveniles (N = 3.6, Median = 3). However, these differences were not significant (Mann-Whitney U test Z = -1.178; P = 0.238). *Oneμ*-13 had the greatest number of alleles (6 alleles) in both groups (naturalized, farmed), followed by *Ots*-9 and *Ots*-10 (5 alleles each). The number of alleles per locus differed with the naturalized salmon having 5 alleles and the farmed specimens 3 alleles at *Ots*-9 and *Ots*-10 and the naturalized samples showing 4 alleles and the farmed fish 5 alleles at *Oneμ*-13. Moreover, the three loci contained alleles that were not shared; these unique alleles are

Table 1

Number of alleles per locus for naturalized and farmed Chinook salmon with total values, loci mean and sample

Número de alelos por locus en el salmón Chinook asilvestrado y de cultivo con valores totales y promedio por locus y tipo de localidad

	Naturalized		Farmed		
	Total	Unique	Total	Unique	Total
Ots-9	5	2	3	0	5
Ots-10	5	2	3	0	5
Oneμ-13	4	1	5	2	6
Mean	4.6	1.6	3.6	0.6	5.3

observed in Table 1. The observed heterozygosity value (Ho) for the combined groups of Chinook salmon was 0.611 ± 0.109, with the naturalized fish presenting a significantly larger value (Ho = 0.699 ± 0.052, Median = 0.70) than the farmed fish (Ho = 0.524 ± 0.061, Median = 0.55) (Mann-Whitney U test Z = -1.963; P = 0.049). The observed and expected heterozygosity values within each group were similar. Most loci fit Hardy-Weinberg equilibrium (Table 2), with the exception of the farmed fish *One*μ-13 sample. When both groups were included, a global fit to the Hardy-Weinberg equilibrium was observed (Fisher = 14.0; P = 0.300). The allelic frequencies showed the same common allele for both groups at the three loci (Table 2). However a high presence of unique alleles was observed for both groups. The naturalized salmon had more unique alleles (4 alleles, 25%) than did the farmed fish (2 alleles, 12.5%). The endogamy index (F_{is}) was slightly greater in the farmed individuals (F_{is} = 0.0457) than in the naturalized samples (F_{is} = -0.0491), which had negative values. Nonetheless, the differences were not significant (Mann-Whitney U test Z = 1.091; P = 0.275). The genotypic disequilibrium values did not show significant differences for each locus and each kind of sample (0.324 < P < 0.818), therefore the mixing of origins between samples was rejected.

The assignment analysis (Fig. 2) indicated that 76.2% of the individuals were correctly classified in their group of origin, with 75% assigned correctly to the naturalized salmon and 77% to the farmed fish. Only between 5% and 3% could be assigned to either of the two origins. The assignment of the individuals based on their multi-locus genotypes showed few individuals distributed close to and around the diagonal and low likelihood values. This allowed the specimens, for the most part, to be assigned to their groups of origin. The bottleneck analysis performed on the naturalized group did not reveal significant values (P = 0.125). Some population reductions were observed, but these were not significant.

Finally, the global analysis of genetic heterogeneity revealed a medium F_{st} value (0.057), thereby establishing a genetic differentiation between the naturalized and farmed individuals. A significant genetic divergence was also found (Fisher, P = 0.001) based on the allelic distribution between both analyzed salmon groups (Table 3), explained mainly by the *Ots*-9 locus.

Table 2

Allelic frequency of the three microsatellite loci used. The number of individuals analyzed (N), Hardy-Weinberg equilibrium fit (PHW), and coefficient of endogamy (F_{is}) are indicated for the farmed and naturalized Chinook salmon samples

Frecuencia alélica de los 3 loci microsatelitales utilizados. Se indica número de individuos analizados (N), ajuste al equilibrio de Hardy-Weinberg (PHW) y coeficiente de endogamia (F_{is}), para las muestras de salmón Chinook de cultivo y asilvestrado

LOCUS								N	PHW	F_{is}
Ots-9	Alleles	104	105	106	107	109				
	Naturalized	0.050	0.550	0.100	0.225	0.075		20	0.325	-0.088
	Farm	0.000	0.545	0.000	0.023	0.432		22	0.811	0.141
Ots-10	Alleles	122	124	126	127	128				
	Naturalized	0.025	0.200	0.275	0.025	0.475		20	0.532	-0.115
	Farm	0.000	0.205	0.114	0.000	0.682		22	0.727	-0.113
Oneμ-13	Alleles	178	180	186	188	190	192			
	Naturalized	0.000	0.000	0.118	0.324	0.441	0.118	17	0.552	0.069
	Farm	0.048	0.048	0.000	0.190	0.570	0.143	21	**0.016**	0.091

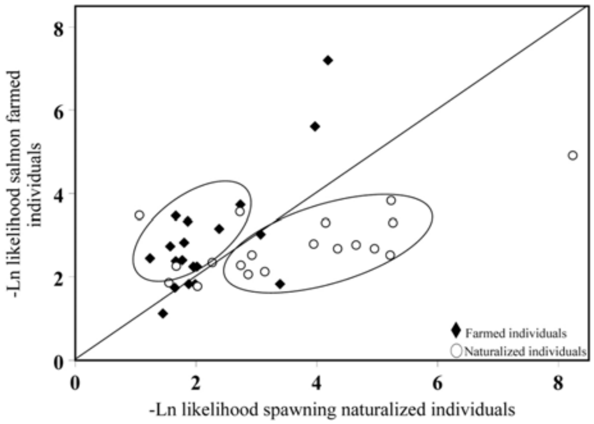

Figure 2

Assignment test based on the individual multi-locus genotype of naturalized and farmed Chinook salmon

Prueba de asignaciones en base al genotipo multilocus individual de salmones Chinook asilvestrados y de cultivo

Table 3

Genetic differentiation and F_{st} values for naturalized and farmed Chinook salmon individuals, *O. tschawytscha* indicating the value of probability (*P*) and Standard Error (SE)

Valores de diferenciación genética y F_{st} para individuos asilvestrados y de cultivo de salmón Chinook *O. tschawytscha,* indicando el valor de probabilidad (*P*) y error estándar (ES)

LOCUS	F_{st}	*P*	SE	Allele n°
Ots-9	0.115	**0.001**	0.001	5
Ots-10	0.038	0.105	0.003	5
Oneμ-13	0.012	0.066	0.002	6
Mean	0.057	**0.001**	0.001	16

Discussion

Our results for naturalized and farmed Chinook salmon are genetically divergent and the naturalized population has a higher percentage of unique alleles than the farmed population does. Thus, the farmed population cannot be genetically linked to the naturalized population and so the fish sampled in the river could not have originated from the farmed sampled population. The genotypic disequilibrium analysis, which is used to determine mixed origins, is not mixed and, therefore, the absence suggests that probably the naturalized populations are not individuals which escaped from current salmon farming sites. Future studies should look into the genetic composition of salmon in other rivers of the Chilean Patagonia where releases have been described as being successful (Di Prinzio 2001, Soto *et al*. 2006, 2007) to construct a history of the colonization process of this species in the rivers of southern Chile.

This work provides the first record of genetic diversity levels for introduced Chinook salmon in Chile's rivers. Genetic variability (more alleles, higher heterozygosity) was greater in the group of naturalized individuals than farmed individuals, which coincides with results obtained for natural Chinook populations of in the northern Hemisphere (Banks *et al*. 2000, Heath *et al*. 2002, Williamson & May 2005) in which high genetic variability was detected through either follow-up of genetic variation over time (Williamson & May 2005), an association with reproductive characters (Heath *et al*. 2002), or the genetic characterization of natural populations (Banks *et al.* 2000). The number of alleles observed per locus was similar to that yielded in the same loci for Chinook in California, where *Ots*-10 had between 2 and 5 alleles and *Oneμ*-13 had 5 alleles (Banks *et al.* 1999, 2000). However, *Ots*-9 has been reported to have 3 alleles per locus (Banks *et al.* 1999, 2000), which is similar to our results for farmed individuals, but different from the naturalized population, which presents 5 alleles. Local biological processes of differentiation (differential selection), which have been taking place in the naturalized populations since their settlement in Chilean waters, may explain this slight differentiation. The Chinook individuals found in the fish farming sites may have been artificially selected for certain characteristics which yielded in a lower number of alleles from bottlenecking.

In addition, the observation of a greater number of alleles in this 'founded' Chilean population in relation to the populations of the northern Hemisphere may be due to: a) multiple population events with individuals from various populations (Holland 2001, Kolbe *et al.* 2004, Therriault *et al*. 2005); b) differential selection processes between the habitats of both hemispheres (Lemaire *et al*. 2000, Lee 2002, Therriault *et al*. 2005); and c) mixing of individuals between those originating in the naturalized population and recent escapees from platform-cages. The assignation values at each site of origin, which indicate a tendency to segregation and, therefore, a genetic divergence between the two samples, revealed that only 3 to 5% of the individuals can be assigned to either of the two origins, suggesting the low possibility that the naturalized population originated from escaped individuals. Given the high variability values, these populations are highly adapted to the new colonized environment. Future studies are planned that will help us understand the colonization of this or other introduced species in the rivers of the Chilean Patagonia by evaluating more loci and their variation over time.

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