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The invasion of Patagonia by Chinook salmon (*Oncorhynchus tshawytscha*): inferences from mitochondrial DNA patterns

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Abstract The Chinook salmon *Oncorhynchus tshawytscha*, which was introduced deliberately in Chile four decades ago for sport fishing and aquaculture, represents a rare example of a successful translocation of an anadromous Pacific salmon into the southern Hemisphere, offering a unique opportunity to examine the role of introduction history and genetic variability in invasion success. We used historical information and mitochondrial displacement loop sequences (D-loop) from seven colonized sites in Chile and Argentina and from native and naturalized Chinook salmon populations to determine population sources and to examine levels of genetic diversity associated with the invasion. The analysis revealed that the Chinook salmon invasion in Patagonia originated from multiple population sources from northwestern North America and New Zealand, and admixed in the invaded range generating genetically diverse populations. Genetic analyses further indicated that the colonization of new populations ahead of the invasion front appear to have occurred by noncontiguous dispersal. Dispersal patterns coincided with ocean circulation patterns dominated by the West Wind Drift and the Cape Horn Currents. We conclude that admixture following multiple introductions, as well as long-distance dispersal events may have facilitated the successful invasion and rapid dispersal of Chinook salmon into Patagonia.

Keywords Human-mediated invasions · Exotic salmonids · Multiple introductions · Admixture · Long-distance dispersal

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Introduction

Patagonia, at the southern end of South America (39°–56°S), is a vast territory surrounded by the Pacific (to the west) and Atlantic (to the east) Oceans, which supports some of the last unpolluted freshwater ecosystems on Earth. Patagonia exhibits relatively low species richness and high levels of endemism (Dyer 2000; Pascual et al. 2002; Cussac et al. 2009; Habit et al. 2012), which provide ideal conditions for the introduction of semiaquatic (e.g., mink and beaver) and aquatic exotic species, including trout and salmon. Salmonids were widely translocated into Patagonian basins from their native ranges in the Northern Hemisphere for both recreational and aquaculture purposes. Most attempts to transplant anadromous species, which breed in fresh water and migrate to the ocean to

feed, to locations around the world have failed. However, several anadromous salmonids have been particularly successful in Patagonia (Pascual and Ciancio 2007). Established populations of anadromous rainbow *Oncorhynchus mykiss* and brown trout *Salmo trutta* were reported in Atlantic rivers of Southern Patagonia at the beginning of the twentieth century, and Chinook salmon *Oncorhynchus tshawytscha*, which was more recently introduced, is actively colonizing both Atlantic and Pacific river basins throughout the region (Ciancio et al. 2005; Correa and Gross 2008; Fernández et al. 2010).

The successful establishment of Chinook salmon in Patagonian rivers began in the late 1970s as a consequence of escapees from fish farms in Chile. Most aquaculture efforts were directed at breeding Atlantic *Salmo salar* and Coho salmon *Oncorhynchus kisutch*. However, Chinook salmon, the species with the least introduction effort in Patagonia was shown to be the most successful at colonizing glacial-fed, cold water Pacific and Atlantic river basins (Soto et al. 2007; Pascual et al. 2009). This is the second example of successful introduction and spread of Chinook salmon in the Southern Hemisphere, following colonization of New Zealand streams from plantings performed during the late 1800s (Quinn et al. 2001), and underlies the remarkable evolutionary potential of this species for colonization, establishment, and subsequent range expansion into new habitats (Ciancio et al. 2005; Correa and Gross 2008).

Chinook salmon exhibit wide variability in life history traits, a characteristic influenced by genetic and environmental factors (Healey 1991; Quinn et al. 2001) that may result in increased invasive potential. Debate is ongoing to establish if this variation provided Chinook salmon with the ancestral capacity to invade novel habitats (i.e., pre-adaptation), and/or if the variation resulted from rapid selective responses to local conditions (i.e., local adaptation) (Ciancio et al. 2005; Correa and Gross 2008). Recipient community attributes, such as suitable environmental conditions and low species diversity (e.g., few predators and competitors), and the unique characteristics of Patagonian aquatic ecosystems have also been suggested as responsible for invasive success (Pascual et al. 2002; Correa and Gross 2008; Schröder and Garcia de Leaniz 2011; Habit et al. 2012).

As with many other organisms, the role of genetic variation in the successful colonization, dispersal, and adaptation of Chinook to novel habitats has received far less attention than other factors. The capacity of a population to respond to selection is proportional to the level of genetic variation, which in turn is affected by the number of founders, or the number of introduction events. This led to a hypothesis that any loss in genetic variability associated with bottlenecks or founder effects during the natural colonization of a new habitat may compromise the adaptive potential of a population to new environments (Sakai et al. 2001). However, studies of human-mediated introductions usually report successful invasions exhibiting similar or augmented levels of genetic variation compared with native populations which are attributed to the introduction of large numbers of individuals, multiple founding population of diverse origins, or admixture (e.g., Astorga et al. 2008; Consuegra et al. 2011). Through admixture, large levels of variation can result in new genetic combinations associated to novel physiological characteristics, which may facilitate rapid adaptation to novel environments (Kolbe et al. 2004, 2008; Dlugosch and Parker 2008; Hänfling 2007).

Determining the attributes responsible for species' invasion success is challenging. Chinook salmon clearly demonstrate increased success at colonizing and dispersing in new environments relative to other anadromous salmonids. One means to investigate the influence of different processes that affect invasion success is to study the invasive species' introduction history and the level of genetic variation of invasive versus native populations (e.g., Le-Roux et al. 2011). Consequently, it may be possible to determine the likely origins, the number of introduction events, and the structure and connectivity of the invasive populations. Introduction history and the invaders' genetic composition can subsequently be bridged with invasion success, aiding in understanding the mechanisms that facilitate the establishment and dispersal of non-native species in newly colonized areas (Wares et al. 2005). In the present study, patterns of genetic diversity among native and introduced Chinook salmon populations were compared using the mitochondrial control region (D-loop) to reconstruct the invasion origins and dispersal patterns of this species.

Materials and methods

Introduction history

Review works of Basulto (2003) and Correa and Gross (2008) were used to obtain Chinook salmon introduction data into Chile and Argentina. We also reviewed unpublished base-line data collected from two National Fisheries Administration Offices from Chile: Subpesca (Subsecretaría de Pesca, http://www.subpesca.cl/), and Sernapesca (Servicio Nacional de Pesca, http://www.sernapesca.cl/). These records indicated the earliest attempts to introduce Chinook salmon in Chile dated back to 1886 from Paris, France (from individuals native to California), and 1924 and 1930, from California, but the efforts were unsuccessful. Additional imports were not reported for at least half a century. However, with the onset of the commercial salmon industry during the 1980s, salmon imports increased considerably. Chinook salmon from the Cowlitz River, a

tributary of the lower Columbia River basin in Washington State, USA, and one stock derived from the Kalama River, also a tributary of the lower Columbia River basin from the University of Washington Hatchery, were introduced on several occasions for ocean ranching in the Chiloé area near Puerto Montt, Chile (Fig. 1a). From 1982 to 1988, male and female gametes from these returns along with Chinook eggs from the University of Washington were used to run a ranching program in the southern channels of Chile's XII Region (49°–56°S), first based at the Santa María (54°S), and later at the Prat (51°S) River (Fig. 1a). Following 1987, additional Chinook salmon from the Oregon coast, Puget Sound in Washington State, and the Vancouver area in British Columbia (Canada) were imported to the X Region (39°–44°S) for experimental net pen rearing. By 1991, Chilean aquaculture converted entirely to ocean net pens and was performed almost exclusively in northern localities along the X and XI Regions (44°–49°S), which imported and reared stocks derived from the Vancouver and Puget Sound areas. Additional strains from commercial stocks were introduced and from New Zealand (of California origins) (Fig. 1b). Chinook salmon imports into Chile ceased during the 2000s.

Beginning in the early 1980s, free-ranging Chinook salmon were recorded in several Pacific basins in proximity to the X and XII Regions, the primary introduction sites (Correa and Gross 2008). The species was also reported in the headwaters of two Pacific basins in Argentina: the Corcovado and Futaleufú Rivers (Grosman 1992) (Fig. 1a). Concurrently, stray fish returns were recorded in the Caterina River (50°S), a small river at the Santa Cruz River headwaters, which drains into the Atlantic Ocean (Ciancio et al. 2005; Becker et al. 2007). During the 1990s, salmon production increased as a result of net pen farming. Reports of Chinook salmon originating from aquaculture facilities continued, with strays occurring into several Pacific outlet rivers in Chile and Argentina from 40°S to 45°S (Basulto 2003; Soto et al. 2007; Correa and Gross 2008; Di Prinzio and Pascual 2008) (Fig. 1b). Documentation of Chinook salmon strays rapidly intensified through the end of the 20th into the beginning of the 21st centuries, including Chilean Tolten (39°S) and Valdivia (40°S) Basin Rivers to the north, the Baker (47°S), Pascua (48°S), and Serrano (51°S) Rivers south of 45°S (Correa and Gross 2008), and the Beagle Channel Rivers (54°S) in Tierra del Fuego (Fernández et al. 2010). Most recently, local fishermen have reported Chinook salmon in the Grande (53°S) and Gallegos Rivers (51°S), two Atlantic basins famous for world-class sport fishery of sea-run brown trout, and in the De las Vueltas River (49°S) in the headwaters of the Santa Cruz River (Fig. 1).

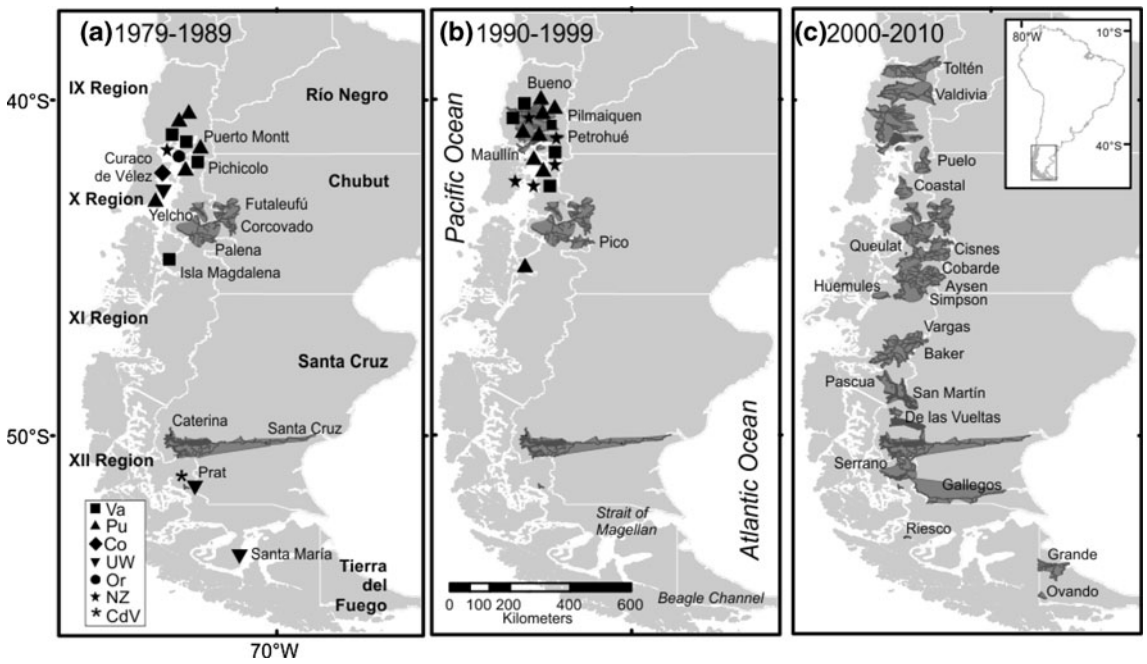

Fig. 1 Introduction and colonization history of Chinook salmon in Chile and Argentina (based on Correa and Gross 2008). The three panels indicate different time periods since the first introduction to Chile. *Black symbol* in panels a and b designate per site stockings from a different geographic source: Vancouver area in British Columbia (Va, *square*), Puget Sound area in Washington State (Pu, *up triangle*), University of Washington (UW, *down triangle*), Cowlitz River in Washington State (Co, *diamond*) Oregon Coast (Or, *circle*), New Zealand (NZ, *star*), and Curaco de Vélez (CdV, *asterisk*). The *shaded areas* are basins, with rivers and lakes shown in a *darker shade*, where free-ranging and spawning individuals have been recorded (names of colonized basins only shown when first noted)

Sampling and DNA techniques

Chinook salmon populations were sampled between January and March 2005 through 2009 from seven major Chilean and Argentinean Patagonia basins, including two original introduction sites: the Cobarde, a tributary of the Simpson (44°S) and Prat (51°S) Rivers in Chile, and five colonized rivers, including the Vargas, a tributary of the Baker (47°S) and Serrano (51°S) Rivers in Chile, and Corcovado (43°S), a tributary of the Palena River, which flows into the Pacific Ocean, Caterina River (50°S) flowing into the Atlantic Ocean, and the Ovando River (54°S) emptying into the Beagle Channel in Argentina (Fig. 2c). Gillnetting, carcass collection, and angling were used in several stations along the watershed to obtain samples. Tissue samples were also collected from 25 fish from a small hatchery located at Pichicolo, near Puerto Mont in the X Region (42°S), which maintains a local Chinook salmon broodstock originally developed from Washington State stocks.

Tissue samples were preserved in 95 % ethanol and DNA was extracted following standard protocols (Sambrook and Russell 2001). PCR was performed to amplify a highly variable segment of the mtDNA (D-loop) control region using the following two primers: T07 (5′-CTTAACTCCCAAAGCTA-3′) (designed by C. Riva Rossi and E. Lessa, Universidad de la República, Montevideo, Uruguay), and P2 (5′-TGTTAAACCCCTAAACCAG-3′, Nielsen et al. 1994). PCR followed the protocol in Nielsen et al. (1994). Amplification yielded 954 base pairs (bp) of high quality sequences from 141 individuals. Amplified DNA templates were purified with the GENECLEAN Purification Kit (Q BIOgene, Carlsbad, CA), and 20 ng of purified PCR product was used in cycle sequencing reactions following ABI PRISM BigDye Terminator protocols (Applied Biosystems, Foster City, CA). Forward and reverse sequences were visualized on an ABI PRISM 3130 automated sequencer at the Centro Nacional Patagonico DNA Sequencing Laboratory and aligned with the MEGA v.5 software (Tamura et al. 2011). Sequences were imported into DNASP version 5 (Librado and Rozas 2009) to identify unique haplotypes and subsequently deposited in GenBank under the accession numbers shown in Table 1. In this study, our 954-bp haplotypes were designated on the basis of homology to published sequences. The standardized nomenclature for short haplotypes (170-bp) in Chinook salmon followed the TSAX format (where X is any integer designating the specific haplotype), and longer haplotypes (414-bp) included the name of short haplotypes that comprised the long haplotype, plus a haplotype-specific suffix (e.g., TSA1A is a long haplotype that includes the short TSA1 haplotype). Reported sequences from our study included an additional haplotype-specific suffix determined by observation order (e.g., longer haplotypes TSA10.1 to TSA10.3 comprise the published TSA10 haplotype, Table 1).

Genetic analysis

The origins of introduced populations were determined using Chinook salmon published sequence data from across the species native and naturalized ranges: California (Nielsen et al. 1994, 1998; Williamson and May 2007), Alaska to California (Martin et al. 2010) and New Zealand (Quinn et al. 1996). We also included short sequences previously recovered by Becker et al. (2007) from the University of Washington stock. These studies were conducted using smaller D-loop fragments (170-bp: Nielsen et al. 1994; Quinn et al. 1996; Becker et al. 2007; 414-bp: Martin et al. 2010) which were nested within the 954-bp segment of mtDNA we sequenced. Therefore, to compare with the most currently published D-loop haplotypes our sequences were trimmed to 414-bp. To infer whether our sampling efforts were sufficient, we used haplotype estimation curves to estimate haplotype diversity in each range (native, New Zealand and Patagonia) and to quantify the effects of sampling effort on haplotype diversity. Specifically, we used the program ESTIMATES 8.0 (Colwell 2005) in order to estimate how many more haplotypes we would expect to find if the sampling effort were increased, given the existing data and sampling information. Samples were randomized 1,000 times without replacement. The following estimators for the total number of haplotypes to be expected, and their respective confidence intervals (where applicable), were extracted: Chao 1 and Chao 2 (Chao 1987), Jackknife 2 (Smith and van Belle 1984) and Michaelis–Menten (Colwell and Coddington 1994).

With the short 414 bp D-loop segment, we examined patterns of genetic similarity in haplotype frequencies among native and non-native populations using a Multidimensional Scaling (MDS) analysis as implemented in the software R version 2.15 (R Development Core Team, 2012). The non-native population sources were also inferred on the basis of the geographic distribution of haplotypes in the native range and phylogeographic relationships among haplotypes. We used the TCS 1.3 program (Clement et al. 2000) to build a haplotype network (95 % statistical parsimony network). Haplotype networks better illustrate genetic divergence at the intra-specific level, particularly in cases where multiple haplotypes derive from a single ancestral sequence (Templeton et al. 1992).

Population genetic analyses were conducted using the software package ARLEQUIN 3.11 (Excoffier et al. 2005), except where noted. Analyses of introduced populations were performed on data for the full 954-bp long

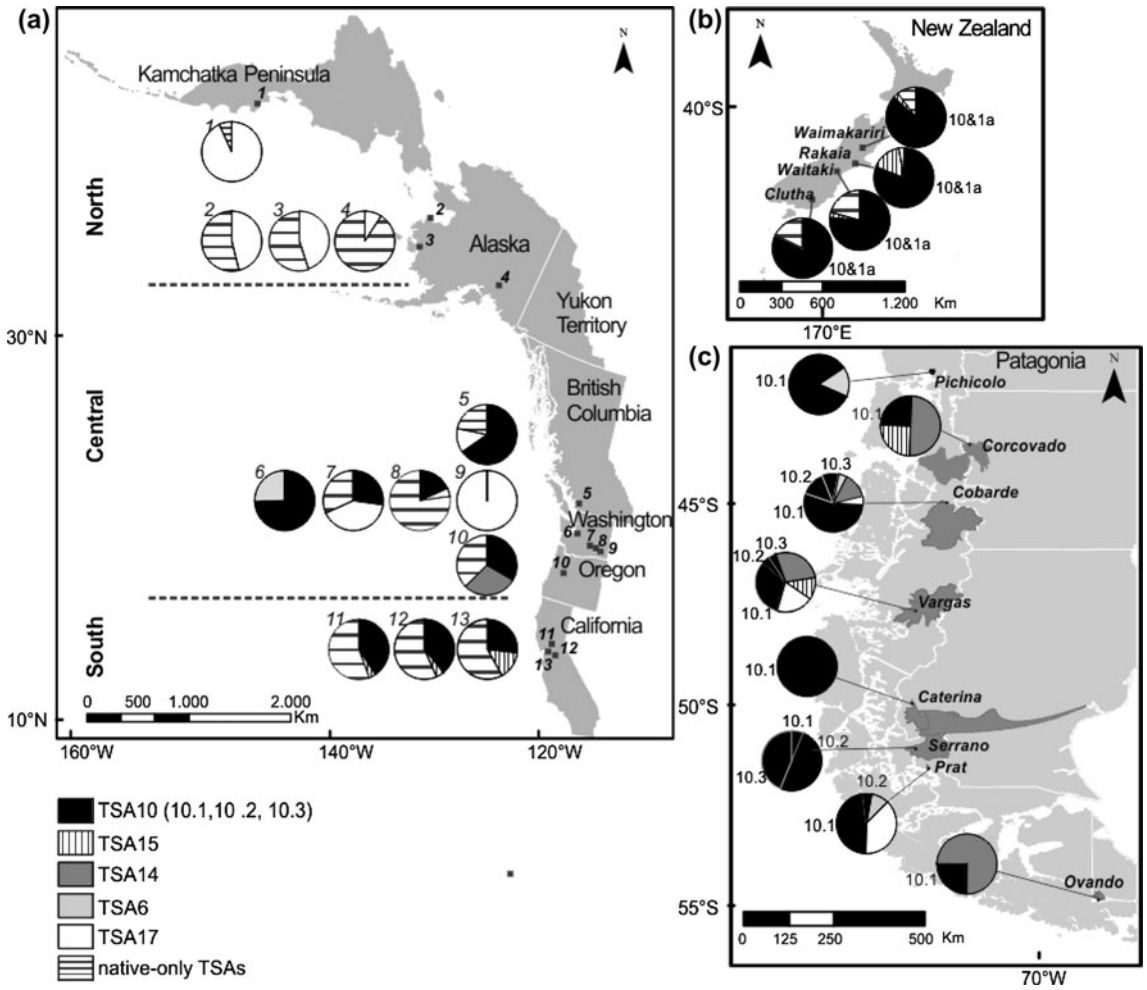

Fig. 2 Locations of Chinook salmon populations analyzed in this study and geographic distribution of mtDNA control region haplotypes from throughout the species' native (**a**), naturalized (**b**), and introduced ranges (**c**) in Patagonia. *Gray-scale* shading designate unique haplotypes and their frequencies within each population. Haplotypes not present in the introduced range were pooled. In **a** the native Chinook salmon range was divided into three regions (north, central, and south) depicted on the *left side* of the figure based on Martin et al. (2010) (see Table 2 for population names). In **b** haplotypes TSA1a and 10 were pooled. In **c** long haplotype frequencies of TSA10.1 to TSA10.3 recovered in this study are represented by a unique shade, but their relative contributions are indicated by their haplotype-specific suffix designation

haplotypes. We estimated haplotype number, gene diversity (h), and nucleotide diversity (π) among locations within the non-native range, which were compared with genetic diversity values reported for native populations using Welch's two-sample *t* test in the statistical program R. We further examined the distribution of genetic variation among and within populations using Analyses of Molecular Variance (AMOVA; Excoffier et al. 1992). We conducted separate AMOVAS accounting for pairwise mutational differences between haplotypes (Φ_{ST}) (Weir and Cockerham 1984; Excoffier et al. 1992) on populations in the native range and for the introduced Patagonia populations and significance was determined with 10,000 permutations. Genetic differentiation between population pairs across the introduced range, with the exception of Corcovado River (where we had a sample size of $N = 4$), was also investigated using an exact test, where haplotype frequencies were compared with a random distribution (Raymond and Rousset 1995). *P* value significance was computed with 10,000 permutations. A modified False Discovery Rate procedure (B-Y FDR, Benjamini and Yekutieli 2001) was applied to correct for multiple pairwise comparisons by adjusting significance levels (Narum 2006). We subsequently tested for a relationship between population differentiation ($[\Phi_{ST}/(1 - \Phi_{ST})]$), and geographical distance (in km) using a Mantel test of isolation by distance (IBD) based on Slatkin's linearized Φ_{ST} values (with 10,000 randomizations).

Results

Origins of introduced Chinook salmon

Five "short" haplotypes were identified when sequences were trimmed down to the 414-bp segment, all of which corresponded to the following published haplotypes: TSA6, TSA10, TSA14, TSA15, and TSA17 (Tables 1, 2; Fig. 2). Haplotype TSA10 (Martin et al. 2010) was detected at all locations across the native range and included shorter haplotype CH1 identified by Nielsen et al. (1994) from California samples, which was recovered by Quinn et al. (1996) in New Zealand samples. It also included shorter haplotypes SC1 and WA1, identical to haplotype CH4 (Nielsen et al. 1994), identified in the Caterina River, the UW stock (Becker et al. 2007) and the Ovando River (Fernández et al. 2010). Haplotypes TSA14, described from Willamette River samples (a major tributary of the Columbia River in Oregon), and TSA15, detected in California samples (Martin et al. 2010), included shorter haplotype TSA3 found in California and New Zealand Chinook samples (Nielsen et al. 1994; Quinn et al. 1996; Nielsen et al. 1998) and in the Ovando River in Argentina (Fernández et al. 2010). Haplotype TSA17 was recovered in populations from Russia to Washington (Martin et al. 2010), and haplotype TSA6 (Nielsen et al. 1998) from California Chinook samples was identical to haplotype WA2 in the UW stock (Becker et al. 2007). This haplotype was also recovered in the Pichicolo sample, a Chilean hatchery founded by Washington State stocks. In aligning the native and non-native mtDNA sequences, a discrepancy was found at position 1,032 (Table 1, based on the base pair positions given by Digby et al. (1992) for *Oncorhynchus mykiss*). In evaluating Nielsen et al. (1994), Becker et al. (2007) assigned an A to the haplotypes at this position for all but the Caterina River and UW samples, where a G was assigned at position 1,032. However, Nielsen et al. (1998) indicated a G at position 1,032, consistent with all other available published sequences for Chinook salmon. All the sequences generated in this study have a G at that position giving not support for a polymorphism.

Haplotype richness (estimated via rarefaction curves of number of haplotypes per number of locations sampled) for each region indicate that both New Zealand and Patagonia introduced ranges have been well-sampled and the detected number of haplotypes is near the asymptote of the predicted total number of haplotype in the system (Fig. 3). In contrast, sampling throughout the native range has not yet reached this asymptote and additional diversity may be discovered with additional sampling (Fig. 3). Extrapolation from the data, with re-sampling, provided estimates of the total number of native range haplotypes between 18 (Chao 1) and 25 (Chao 2) (Table 2), compared to the 17 detected by Martin et al. (2010).

The MDS analysis based on haplotype frequencies indicated the presence of three main distinct clusters (Fig. 4). Among native range populations, Chinook salmon from Russia and Alaska were placed close to Washington populations. The populations from California formed a fairly compact cluster together with naturalized populations from New Zealand and were well separated from the remaining native populations. The non-native populations fell into a third cluster along with the University of Washington stock and the populations from British Columbia and Oregon. Within this cluster, those populations with high frequency of haplotype TSA10 (Cobarde, Prat, Caterina and Serrano Rivers) were closest to the University of Washington stock and British Columbia whereas those with higher frequency of haplotype TSA14 (Vargas, Corcovado and Ovando) were closest to the Willamette River population in Oregon. The 95 % parsimony TCS haplotype network revealed limited phylogeographic structure based on the mtDNA D-loop across the native Chinook salmon range (Fig. 5). Native species populations consisted of a relatively large number of closely related haplotypes. The four most common (TSA 17, TSA 1B, TSA 1A, and TSA10) were detected in the central geographic region of the native range. Two of these haplotypes (TSA 1A and TSA 10) were not identified in the northern region of the range, and the other two haplotypes (TSA 17 and TSA 1B) were not detected from the southern geographic area. Additional haplotypes were found exclusively in the northern (TSA 20 and TSA 21), central (TSA11, 12, 13, 16), or southern regions (TSA 2A and TSA 15) (Martin et al. 2010). Introduced populations exhibited fewer haplotypes than native populations (five vs. 12 for the shorter fragment, respectively) and were distributed in different sectors of the network, with haplotypes identified in different geographic regions within the native range.

Genetic variation and structure within the introduced range

The longer 954-bp fragment resulted in seven distinct haplotypes: TSA10.1 to TSA10.3, TSA6.1, TSA14.1, TSA15.1, and TSA17.1 (see Table 1, Sampled Populations). Haplotype TSA10.1 was the most common (detected at all non-native locations) and represented 54.2 % of the individuals. High TSA10.1 frequency in the introduced area was congruent with the predominance of the haplotype in various locations across the native species range. The second most frequent haplotype was TSA14.1, represented in 13.7 % of the individuals recorded at northern Patagonia localities and the Ovando River. TSA10.2, TSA10.3, TSA6.1, and TSA17.1 haplotypes were found in a frequency ranging from 9.0 to 4.9 % in individuals distributed in northern and

Table 1 GeneBank accession, haplotype name, and variable sites for the mtDNA control region sequences surveyed in Chinook salmon native and introduced populations

Accession no. or reference	Haplotype	203	383	408	649	650	745	779	986	1006a	1019	1021	1032	1033	1050	1081	1089	1099	1130	1134	1136	1139	1147
Shedlock et al. (1992)	HapS	G	C	G	C	C	G	G	A	G	T	C	G	C	T	T	C	A	A	C	C	G	A
Nielsen et al. (1998)	TSA1[a]	?	?	?	?	?	?	?	.	.	.	T	.	.	.	.	.	.	.	.	/-	.	.
	TSA2	?	?	?	?	?	?	?	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.
	TSA3	?	?	?	?	?	?	?	.	.	.	T	.	.	.	.	A	.	.	.	.	.	.
	TSA4	?	?	?	?	?	?	?	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.
	TSA5	?	?	?	?	?	?	?	.	.	.	T	.	.	.	81i[b]	.	.	.	.	.	.	.
	TSA6	?	?	?	?	?	?	?	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	TSA7	?	?	?	?	?	?	?	.	.	.	T	.	.	G	.	A	.	.	.	.	.	.
	TSA8	?	?	?	?	?	?	?	.	.	.	T	.	.	G	.	.	.	.	.	.	.	.
	TSA9	?	?	?	?	?	?	?	.	A	A	T	.	.	G	.	.	.	.	.	.	.	.
EU489727	TSA1a	?	?	?	?	?	A	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
EU489729	TSA1b	?	?	?	?	?	A	A	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
EU489735	TSA2a	?	?	?	?	?	A	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.
EU489732	TSA4a	?	?	?	?	?	A	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.
EU489726	TSA10	?	?	?	?	?	A	.	.	.	.	T	.	.	.	.	.	.	.	.	–	.	.
EU489738	TSA11	?	?	?	?	?	A	.	.	.	.	T	.	.	.	.	.	.	.	A	–	.	.
EU489724	TSA12	?	?	?	?	?	A	.	.	.	.	T	.	.	.	.	.	.	.	.	–	.	.
EU489737	TSA13	?	?	?	?	?	A	.	.	.	.	T	.	.	.	.	.	.	.	.	–	A	.
EU489725	TSA14	?	?	?	?	?	A	.	.	.	.	T	.	.	.	.	A	.	.	.	–	A	.
EU489736	TSA15	?	?	?	?	?	A	.	.	.	.	T	.	.	.	.	A	.	.	.	–	.	.
EU489734	TSA16	?	?	?	?	?	A	.	.	.	.	T	.	.	.	.	.	G	.	.	.	.	G
EU489730	TSA17	?	?	?	?	?	A	A	.	.	.	T	.	.	.	.	.	.	C	.	.	.	.
EU489739	TSA18	?	?	?	?	?	A	A	.	.	.	T	T	.	.	.	.	.	C	.	.	.	.
EU489731	TSA19	?	?	?	?	?	A	A	G	.	.	T	.	.	.	.	.	.	C	.	.	.	.
EU489728	TSA20	?	?	?	?	?	A	A	.	.	.	T	.	.	.	.	A	.	C	.	.	.	.
EU489733	TSA21	?	?	?	?	?	A	A	.	.	.	T	.	T	.	.	A	.	C	.	.	.	.
EF531713	SC1	?	?	?	?	?	?	?	.	.	.	T	.	.	.	.	.	.	.	.	–	.	.
EF531711	WA2	?	?	?	?	?	?	?	.	.	.	.	.	.	.	.	.	.	.	.	–	.	.
EF531712	WA1	?	?	?	?	?	?	?	.	.	.	T	.	.	.	.	.	.	.	.	–	.	.
JX975268	TSA10.1	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	.	.	.	.	/-	.	.
JX975269	TSA10.2	.	T	A	T	T	A	.	.	.	.	T	.	.	.	.	.	.	.	.	/-	.	.
JX975270	TSA1.03	.	T	.	T	T	A	.	.	.	.	T	.	.	.	.	.	.	.	.	/-	.	.
JX975274	TSA14.1	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	A	.	.	.	–	A	.
JX975275	TSA15.1	.	T	.	.	.	A	.	.	.	.	T	.	.	.	.	A	.	.	.	–	.	.
JX975276	TSA6.1	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	–	.	.

Table 1 continued

Accession no. or reference	Haplotype	203	383	408	649	650	745	779	986	1006a	1019	1021	1032	1033	1050	1081	1089	1099	1130	1134	1136	1139	1147
JX975277	TSA17.1	.	T	.	.	.	A	A	.	.	.	T	.	.	.	.	.	.	C	.	.	.	.

Nucleotide (nt) numbers corresponds to those given in Digby et al. (1992) for *O. mykiss*. The "–" represents a gap, "?" represents a missing nt and "." matches the nucleotide in the first sequence

[a] TSA1 from Nielsen et al. (1998) is equivalent to CH1 and CH4 described in Nielsen et al. (1994), which differ by a base change at position 1,136 (a C in CH1 and a deletion in CH4)

[b] An 81-base-pair insertion was found in Chinook salmon

Table 2 Mean asymptotic values of four extrapolation estimators and their confidence intervals (CI), where applicable, as applied to the mtDNA Chinook salmon data

	Hobs	Chao 1	Chao 2	Jack 2	MM means
NA	17				
	Mean	18.25	25.00	24.98	21.86
	95 % CI lower bound	16.27	17.52	NA	NA
	95 % CI upper bound	35.04	69.28	NA	NA
NZ	5				
	Mean	5.00	5.00	3.81	6.18
	95 % CI lower bound	5.00	5.00	NA	NA
	95 % CI upper bound	5.00	6.12	NA	NA
PAT	5				
	Mean	5.00	5.00	6.25	5.35
	95 % CI lower bound	5.00	5.00	NA	NA
	95 % CI upper bound	5.00	5.88	NA	NA

NA North American samples, *NZ* New Zealand samples, *PAT* Patagonia samples

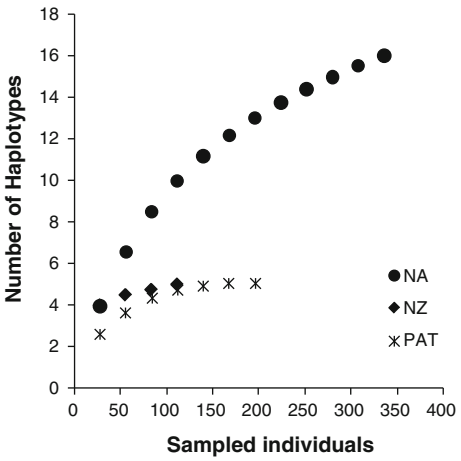

Fig. 3 Rarefaction curves of observed haplotype diversity in mtDNA data detected at each range: *NA* North America, *NZ* New Zealand, and *PAT* Patagonia

southern localities. Finally, haplotype TSA15.1 was represented in 2.8 % of the individuals and was recorded at the Corcovado and Vargas Rivers (Table 3; Fig. 2).

Average non-native population gene diversity was higher ($h = 0.656 \pm 0.109$; excluding the Caterina River sample, which was fixed for the TSA10.1 haplotype) than that reported for the native Chinook salmon range [$h = 0.592 \pm 0.070$; excluding the Tucannon River sample, from the Washington State, which was fixed for the TSA10 haplotype,

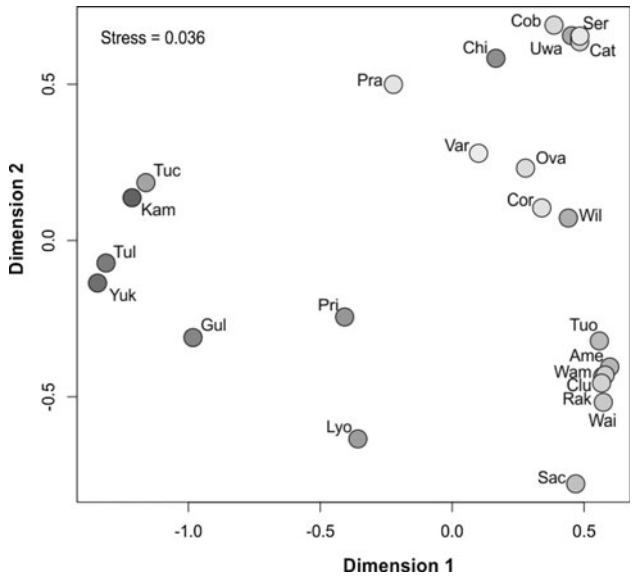

Fig. 4 MDS plot based on the Manhattan distance constructed from mtDNA haplotype frequencies for Chinook salmon populations from the native, New Zealand, and introduced ranges. Population similarity is indicated by *gray-scale shading*. Populations codes are: Native range: Kam: Kamchatka, RU; Yuk: Yukon, AK; Tul: Tuluksak, AK; Gul: Gulkana, AK; Chi: Chilliwack River, BC; UWa: University of Washington, WA; Pri: Priest Rapids, WA; Lyo: Lyons Ferry, WA; Tuc: Tucannon, WA; Wil: Willamette River, OR; Ame: American River, CA; Tuo: Tuolumne River, CA; and Sac: Sacramento River, CA. New Zealand: Clu: Clutha River, NZ; Wai: Waitaki River, NZ; Rak: Rakaia River, NZ; and Wam: Waimakariri, NZ. Introduced range (Patagonia): Cob: Cobarde River, CH; Cor: Corcovado River, AR; Var: Vargas River, CH; Ser: Serrano River, CH; Pra: Prat River, CH; and Cat: Caterina River, AR

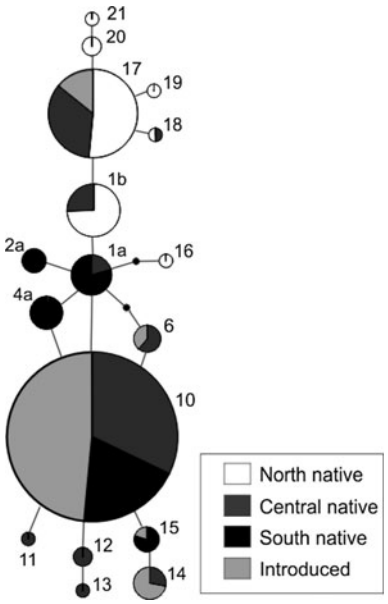

Fig. 5 Ninety-five percent statistical parsimony haplotype network for Chinook salmon based on mtDNA control region short haplotypes (414-bp). *Circle* size is proportional to the number of individuals. Each line represents a single mutation; *black dots* represent inferred non-sampled or extinct haplotypes

data from Martin et al. (2010)], but this difference was not significant (one-tailed t test $= -0.625$, $P > 0.05$). However, non-native populations exhibited a lower yet non-significant (one-tailed t test $= 0.172$, $P > 0.05$, Table 3) average nucleotide diversity ($\pi = 0.0014 \pm 0.0007$) than native populations ($\pi = 0.0018 \pm 0.0007$). In the introduced range, increased genetic diversity was observed at rivers close to or at original points of introduction, including the Corcovado, Vargas, Cobarde, Prat, and Serrano Rivers, ranging from 0.833 to 0.592, compared to less diverse peripheral locations (Ovando and Caterina, $h = 0.436$ and 0.000, respectively). The Pichicolo sample exhibited low levels of genetic diversity, characteristic of hatchery stocks. Nucleotide diversity followed the same trend (Table 2). AMOVA analyses indicated that in the native region, 53.6 % ($P < 0.0001$) of the total genetic variation occurred among populations, with the remainder within populations. In contrast, introduced populations in Patagonia exhibited lower among population genetic variation (36.9 %, $P < 0.0001$) and higher within-population variation (64.1 %).

Despite higher within-population variation, significant population subdivision was found among introduced Chinook salmon populations in Chile and Argentina ($\Phi_{ST} = 0.369$, $P < 0.001$). In Φ_{ST} pairwise comparisons, the following three population tests were non-significant: Cobarde versus Vargas, Vargas versus Ovando, and Cobarde versus Ovando. In Cobarde versus Ovando, the lack of Φ_{ST} significance might be due to small sample size, whereas the remaining population-pairs showed significant differentiation with Φ_{ST} values ranging from 0.111 to 0.872 ($P < 0.017$) (Table 4). The most noteworthy significant pairwise Φ_{ST} comparisons involved the Serrano and Caterina samples. As expected from these results, a relationship between population differentiation and geographical distance among introduced populations was not evident ($r = 0.053$, $P > 0.05$), providing no foundation for IBD.

Discussion

The combined use of historical and mitochondrial DNA data enables the portrayal of inferences regarding the origins and colonization processes of Chinook salmon introduced into Patagonian basins (41°–54°S). Our results also support the hypothesis that multiple introductions resulted in the establishment of genetically diverse populations. Moreover, we found evidence for admixture and genetically novel combinations in several sampled locales. Historical records suggest that Chinook salmon have been repeatedly and intensively introduced to several locations in Chile for at least 30 years, and from as many as six geographically distinct Chinook origins, from the following stocks: Washington State, including the Cowlitz River

Table 3 Descriptive statistics of mitochondrial DNA (mtDNA) control region comparing genetic variation among Chinook salmon populations from introduced and native ranges

	Locality	*n*	20	21	16	19	18	17	1B	1A	10	11	13	12	14	4A	2A	15	6	H	*H*	π	References
Reference populations (414-bp)	1. Kamchatka, RU	31	1				1	28	1											4	0.187 (0.093)	0.0003 (0.0005)	Martin et al. (2010)
	2. Yukon, AK	20	2					9	9											3	0.616 (0.058)	0.0013 (0.0011)	Martin et al. (2010)
	3. Tuluksak, AK	26	5	1				12	8											4	0.680 (0.054)	0.0016 (0.0013)	Martin et al. (2010)
	4. Gulkana, AK	22						2	20											2	0.173 (0.101)	0.0003 (0.0005)	Martin et al. (2010)
	5. Chilliwack River, BC	23			3			3			15	1	1							5	0.561 (0.110)	0.0027 (0.0019)	Martin et al. (2010)
	6. University of Washington (Kalama River), WA	32									32								11	2	n.p.	n.p.	Becker et al. (2007)
	7. Priest Rapids, WA	22				1		9	2	4	6									5	0.749 (0.058)	0.0028 (0.0019)	Martin et al. (2010)
	8. Lyons Ferry, WA	22				1	1	1	11	3	4					1				7	0.723 (0.087)	0.0023 (0.0017)	Martin et al. (2010)
	9. Tucannon, WA	21						21												1	0	0	Martin et al. (2010)
	10. Willamette River, OR	24								2	8			7	7					4	0.743 (0.038)	0.0026 (0.0018)	Martin et al. (2010)
	11. American River, CA	27								8	11					3	4	1		5	0.738 (0.052)	0.0018 (0.0014)	Martin et al. (2010)
	12. Tuolumne River, CA	23								2	9					7	4	1		5	0.747 (0.054)	0.0021 (0.0015)	Martin et al. (2010)
	13. Sacramento River, CA (fall run)	75								20	16					21	8	10		5	n.p.	n.p.	Williamson and May, (2007)
	14. Clutha River, NZ	62								51[a]						11		0		4	n.p.	n.p.	Quinn et al. (1996)
	15. Waitaki River, NZ	34								26[a]						7		1		4	n.p.	n.p.	Quinn et al. (1996)
	16. Rakaia River, NZ	37								30[a]						1		6		4	n.p.	n.p.	Quinn et al. (1996)
	17. Waimakariri, NZ	39								34[a]						3	1	1		5	n.p.	n.p.	Quinn et al. (1996)

Table 3 continued

	Locality	*n*	20	21	16	19	18	17	1B	1A	10	11	13	12	14	4A	2A	15	6	H	*H*	π	References
Sampled populations (954-bp)	18. Cobarde River, CH	22						1			$12^{10.1}$ $3^{10.2}$ $2^{10.3}$				3				1	6	0.636 (0.105)	0.0017 (0.0012)	This study
	19. Corcovado River, AR	4									$1^{10.1}$				2			1		3	0.833 (0.222)	0.0012 (0.0012)	This study
	20. Vargas River, CH	25						5			$8^{10.1}$ $1^{10.2}$ $1^{10.3}$				7			3		6	0.793 (0.042)	0.0021 (0.0014)	This study
	21. Serrano River, CH	16									$1^{10.1}$ $8^{10.2}$ $7^{10.3}$									3	0.592 (0.066)	0.0008 (0.0007)	This study
	22. Prat River, CH	21						8			$10^{10.1}$ $1^{10.2}$								2	4	0.647 (0.065)	0.0015 (0.0011)	This study
	23. Caterina River, AR	20									$20^{10.1}$									1	0	0	This study
	24. Ovando River, AR	8									$2^{10.1}$				6					2	0.436 (0.133)	0.0009 (0.0008)	This study
	25. Pichicolo Hatchery, CH	25									$21^{10.1}$								4	2	0.280 (0.101)	0.0030 (0.0001)	This study

n, number of samples; H, number of haplotypes, *h* (SD), gene diversity and its standard deviation, π (SD), nucleotide diversity and its standard deviation, n.p., analysis not performed. For haplotype TSA10 the superscript indicates the longer haplotype type. Localities: RU, Russia; AK, Alaska; BC, British Columbia; WA, Washington; OR, Oregon; CA, California; NZ, New Zealand; CH, Chile and AR, Argentina. Haplotype information for Russia, Alaska, British Columbia, Washington, Oregon and California based on Martin et al. (2010) (414-bp); for the Sacramento River based on Williamson and May (2007) (237-bp) and for New Zealand based on Quinn et al. (1996) (170-bp)

[a] In Quinn et al. (1996) the base change differentiating haplotypes TSA1a and TSA10 (CH1 and CH4, respectively, in that study) was difficult to score consistently in the NZ fish, therefore they pooled mtDNA types 1 and 4

Table 4 Results of pairwise comparisons of non-native Chinook salmon populations in Chile and Argentina

	Cobarde	Vargas	Serrano	Prat	Caterina	Ovando
Cobarde	–	610	1,020	960	2,670	1,525
Vargas	0.057	–	610	550	1,525	1,115
Serrano	**0.442***	**0.531***	–	60	1,035	625
Prat	**0.160***	**0.106***	**0.597****	–	975	565
Caterina	**0.111***	**0.196****	**0.852****	**0.462***	–	860
Ovando	0.319	0.109	**0.791***	**0.359***	**0.872***	–

Φ_{ST} values are given below the diagonal and the geographic distances between river mouths (km) above the diagonal. Bold tests and levels of significance after false discovery rate correction ($\alpha = 0.003$) are marked with * $P \leq 0.05$ and ** $P \leq 0.01$

(introduced from 1978 to 1983), UW (1982–1989), and the Puget Sound (1987–1997); populations from Oregon (1987–1988); British Columbia; and New Zealand (introduced from 1988 to 2000). The mtDNA data largely corroborated historical records, detecting a close affinity among Chinook salmon stocks from the University of Washington and British Columbia (TSA10, TSA6 and TSA17) and the Cobarde, Prat, Caterina and Serrano populations, whereas closest genetic affinities were detected among Oregon (TSA14), and to a lesser extent, New Zealand (TSA15) with the Vargas, Ovando and Corcovado Rivers populations. Previous studies based on historical records and field data alone contended that naturalized populations of Chinook salmon in Patagonia were likely derived from ocean ranching operations in Chile (Correa and Gross 2008). However, our results indicate that invading Chinook salmon have likely originated from both early ocean ranching and recent net pen operations in Chile.

Genetic patterns observed in introduced populations that exhibited haplotypes with distantly disjunct distributions in the native range co-occur in Patagonia. This is congruent with the hypothesis that introduced populations have multiple source origins. Moreover, at least four of the seven introduced populations sampled in this study (Cobarde, Corcovado, Vargas, and Prat) exhibited haplotypes that originated from more than one distinct native source, reflecting genetic mixing from previously isolated lineages. For example, haplotypes sampled from Chilean locations north of 47°S (TSA6, 10, 14, 15, and 17) suggested ancestral contributions from all putative stocks, whereas the Prat River haplotype composition (TSA6, 10, and 17) indicated ancestral contributions derived from fewer sources, primarily the Washington State. When we consider historical records, we cannot rule out secondary colonization from additional sources introduced at Curaco de Vélez, such as British Columbia. Due to the overall absence of genetic diversity, molecular data were not useful to clarify Chinook salmon origins in the Caterina River. However, historical data (reports of first sightings soon after the initiation of the ranching experiments on the Prat River) lead to support the hypothesis proposed by Ciancio et al. (2005) that the invasion was likely the result of imports into southern Chile in the early 1980s.

As expected from admixture following multiple introduction events, genetic diversity in the non-native Chinook salmon populations sampled in this study was not significantly lower than in native source populations, despite a trend towards slightly lower nucleotide variation. Increased genetic diversity in introduced relative to native populations have been also observed in invading populations of brown anole lizards *Anolis sagrei* (Kolbe et al. 2004) and of the amphipod *Gammarus tigrinus* (Kelly et al. 2006). Both studies suggested that interbreeding among individuals from different native-range sources caused admixture, which combined among-population genetic variation from multiple genetically differentiated sources to increase genetic variation within introduced populations. The notable loss of genetic diversity in the Caterina River Chinook salmon, however, might result from the interaction between recent (secondary) founder events, which are typical in populations founded from noncontiguous colonization at the extreme edge of an invasion range (Hewitt 1996; Ibrahim et al. 1996), and rapid selection due to strong local adaptation in this population.

Significant population differentiation, but no evidence of IBD or a geographic cline, was observed within our study area, which is to be expected if the invasion expanded gradually in a front-like manner, whereby the most recently invaded location was the source of further invasion. In most cases, genetic distances were low and populations diverged less than would be expected for the distance separating each population. This pattern can be better explained by the introduction of the same Chinook salmon genetic sources to different locations in the invaded range than by progressive expansion and contiguous dispersal. The exception was the Serrano River, which exhibited more genetic divergence than expected, even from the nearby Prat River, located a short geographic distance away (60 km). The two locations are situated

within the Última Esperanza Sound, an inland waterway that empties the Cordillera del Paine. Although hydrological studies have documented a significant net outflow of surface waters, local bathymetry creates a relatively high retention time, which may hamper the exchange between marine and freshwater fauna (Antezana 1999) and explain low Chinook salmon dispersal (particularly smolts) in and out of the Sound. The markedly high levels of genetic differentiation in the Caterina River, the most remote location, suggest that this population arose via geographic isolation and is currently disconnected from the remaining colonizing populations (but see above also for alternative explanations).

We also detected several instances of long distance southward dispersal. For example, haplotype TSA15, introduced from New Zealand to northern Chilean localities (42°S) in the 1990s, was first recorded in the Vargas River in the year 2000, 700 km south of the introduction sites. The presence of haplotypes TSA14 (derived from Oregon) and TSA10.3 (unknown ancestry) in the Ovando and Serrano Rivers, which appear in high frequencies at northern Chilean locales (>1,100 km for the Ovando population) but are absent in the geographically closer Prat population (Fig. 2), is consistent with a hypothesis of a general pattern of southern spread by noncontiguous dispersal.

These results are also congruent with the ocean circulation patterns around southern South America, largely dominated by the cold waters of the westward flowing West Wind Drift, and the southward flowing Cape Horn Current, which would facilitate southward salmon dispersal from Chilean locations into Antarctic convergence waters and further into the Patagonian Shelf in the southwestern Atlantic Ocean (Becker et al. 2007). Other authors have proposed a similar dispersal scenario for rockfishes along the coast of South America via the Humboldt Current and the West Wind Drift current (Eschmeyer and Hureau 1971; Nuñez et al. 2010). As net pen Chinook salmon cultures in Chile moved further south and occupied new watersheds, the risk of exotic Chinook salmon spreading further and colonizing aquaculture-free Patagonian basins, is extremely high (Consuegra et al. 2011; Fernández et al. 2010; Pascual et al. 2009).

In conclusion, our study indicates that the deliberate introduction of Chinook salmon from several founding sources into Patagonia has contributed to the maintenance of high levels of genetic variation within non-native populations, thus avoiding the loss of genetic variation associated with the colonization of new habitats. These high levels of genetic variation may have facilitated the successful establishment of Chinook salmon populations in Patagonia.

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