Environmental Biology of Fishes **69:** 37–50, 2004.

Genetic population structure of chum salmon in the Pacific Rim inferred from mitochondrial DNA sequence variation

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Received 17 April 2003 Accepted 27 April 2003

Key words: mtDNA control region, Pacific salmon, haplotype genealogy, genetic divergence

Synopsis

We examined the genetic population structure of chum salmon, *Oncorhynchus keta*, in the Pacific Rim using mitochondrial (mt) DNA analysis. Nucleotide sequence analysis of about 500 bp in the variable portion of the 5′ end of the mtDNA control region revealed 20 variable nucleotide sites, which defined 30 haplotypes of three genealogical clades (A, B, and C), in more than 2,100 individuals of 48 populations from Japan (16), Korea (1), Russia (10), and North America (21 from Alaska, British Columbia, and Washington). The observed haplotypes were mostly associated with geographic regions, in that clade A and C haplotypes characterized Asian populations and clade B haplotypes distinguished North American populations. The haplotype diversity was highest in the Japanese populations, suggesting a greater genetic variation in the populations of Japan than those of Russia and North America. The analysis of molecular variance and contingency χ^2 tests demonstrated strong structuring among the three geographic groups of populations and weak to moderate structuring within Japanese and North American populations. These results suggest that the observed geographic pattern might be influenced primarily by historic expansions or colonizations and secondarily by low or restricted gene flow between local groups within regions. In addition to the analysis of population structure, mtDNA data may be useful for constructing a baseline for stock identification of mixed populations of high seas chum salmon.

Introduction

Chum salmon, *Oncorhynchus keta*, has the widest natural geographical distribution among all Pacific salmon species in the North Pacific Rim, ranging from Korea and Japan northward to the Arctic coasts of Russia and North America and then southward to Oregon (Salo 1991). Spawning adults, like other Pacific salmons, are

anadromous to the natal river. Such restricted homing behavior will lead geographically distinct populations to partial genetic isolation. Estimation of genetic variation among and within the Pacific Rim populations of chum salmon is therefore important for addressing the population history, the patterns of ocean migration, and the stock composition in high seas aggregations and coastal commercial fisheries.

The genetic variation of chum salmon has been examined by allozyme analysis (Kondzela et al. 1994, Phelps et al. 1994, Wilmot et al. 1994, Winans et al. 1994), which has been used for stock identification of population mixtures in the ocean (Wilmot et al. 1998, Seeb & Crane 1999b). However, analysis of allozymes requires careful collection and handling of tissues (Brown et al. 1979), and resolution of allozymes remains mostly at the regional- to continental-levels (Brown et al. 1979, Wilmot et al. 1998). Moreover, allozyme data are often inadequate for discriminating causal factors of population divergence (Zhivotovsky et al. 1994).

Recently developed molecular techniques are likely to provide a powerful means to observe genetic variation in salmon populations with increased accuracy and resolution (Ferguson et al. 1995). Maternally inherited mitochondrial (mt) DNA has higher sequence variability than most single copy nuclear genes (Brown et al. 1979). The non-coding control region has been recommended for assessing intraspecific genetic variation in the species of interest, because it often has higher variability than the coding regions (Moritz et al. 1987, Meyer 1993), although this situation is not always the case for some whale (Hoelzel et al. 1991) and fish species (Bernatchez et al. 1992, Pigeon et al. 1998) including salmon (Churikov et al. 2001). Thus, analysis of mtDNA has become a method of choice in many phylogenetic, population genetic, and evolutionary studies (Moritz et al. 1987, Meyer 1993).

Previous studies on restricted fragment length polymorphisms (RFLPs) showed low levels of sequence variation in limited mtDNA segments of salmonids including chum salmon (Wilson et al. 1987, Cronin et al. 1993, Park et al. 1993, Bickham et al. 1995). Although a genome-wide RFLP study using a number of restriction enzymes detected a few hypervariable coding regions of mtDNA in chum and other Pacific salmon species (Churikov et al. 2001), such an extensive analysis is not easy to apply for stock identification using a large number of fish. Moreover, allozyme and mtDNA RFLP analyses provided similar estimates in stock identification of mixed fisheries of chum salmon (Seeb & Crane 1999b), although that study did not survey the entire mtDNA genome.

Recently, Sato et al. (2001) detected greater amount of variation in the mtDNA control region by nucleotide sequence analysis than the variation observed by a previous RFLP analyses (Park et al. 1993). This finding indicates an increased potential of mtDNA sequence analysis to estimate the genetic variation of chum salmon populations. In this collaborative study, nucleotide sequencing of the mtDNA control region was conducted to examine its potential use in analyzing the genetic variation and population structure of chum salmon, using more than 2,100 individuals from 48 populations in Japan, Korea, Russia, and North America.

Materials and methods

Samples

Liver, blood, or muscle samples of chum salmon were collected from 1,617 individuals of 36 populations from Japan (four populations), Korea (one population), Russia (10 populations), and North America including Alaska (13 populations), British Columbia (three populations), and Washington (five populations) from 1988 to 2000 (Table 1 and Figure 1). All samples,

Table 1. Sampling locations, date of collection, and the numbers of chum salmon samples (N) used for mtDNA analysis.

	Sampling location	Date of collection	N
Japan			
Hokkaido Island			
1	Chitose River*	14 Oct. 1996	51
2	Tokushibetsu River*	23 Sep. 1997	51
3	Tokoro River (late-run)*	20 Nov. 1998	44
4	Tokoro River (early-run)*	13 Oct. 1999	49
5	Nishibetsu River*	25 Sep. 1997	41
6	Kushiro River	22 Oct. 1998	49
7	Tokachi River*	17 Oct. 1996	46
8	Yurappu River*	17 Nov. 1998	40
Honshu Island			
9	Tsugaruishi River (late-run), Iwate Pref.*	10 Dec. 1997	44
10	Tsugaruishi River (early-run), Iwate Pref.	Oct. 1999	47
11	Otsuchi River, Iwate Pref.*	8 Apr. 1999	49
12	Koizumi River, Miyagi Pref.*	21 Nov. 1996	47
13	Kawabukuro River, Akita Pref.*	18 Nov. 1997	30
14	Gakko River, Yamagata Pref.*	10 Dec. 1996	45
15	Uono River, Niigata Pref.	23–24 Oct. 1996	49
16	Jintsu River, Toyama Pref.	7 Nov. 1995	49

Table 1. (*Continued*)

Sampling location		Date of collection	N
Korea			
17	Namadae River	13 Nov. 2000	46
Russia			
Anadyr			
18	Anadyr River	1990	43
Kamchatka Peninsula			
19	Hairsova River	1993	41
20	Kamchatka River	1991	46
21	Vorovskaya River	1990	32
22	Kol River	1991	44
Sakhalin island			
23	Kalininka River	1994	42
Magadan			
24	Ola River	1990	33
25	Magadan River	1991	37
Nikolaevsk-na-Amure			
26	Amur River	9 Sep. 2000	50
Primorye			
27	Avakumovka River	1994	30
North America			
Northwest Alaska			
28	Salmon River	1991	45
29	Sheenjek River (fall-run)	1992	45
30	Andreafsky River (summer-run)	1993	48
31	Togiak River	1993	49
Alaska Peninsula			
32	Belkofski River	1992	44
Southcentral Alaska			
33	Kizhuyak River	1992	46
34	Olsen Creek	1992	45
Southeast Alaska			
35	Sawmill Creek, Berner's Bay	28 July. 1993	50
36	Long Bay, Chichigof Island	25–26 Aug. 1991	49
37	Whale Bay, Baranof Island	12 Aug. 1993	48
38	Port Beauclerc, Kuiu Island	20 Aug. 1995	45
39	Fish Creek, Portland Canal	25 Sep. 1988	49
40	Disappearance Creek, POW Island	25 Sep. 1998	50
British Columbia			
41	Ecstall River, Skeena River area	12 Sep. 1988	45
42	Bag Harbor, QCI	Mid-Oct. 1989	50
43	Nekite Channel	15 Sep. 1989	33
Washington			
44	Nooksack River	1998	47
45	Quilcene Bay	1998	49
46	Blackjack Creek	1998	50
47	Satsop River	1998	49
48	Hamilton Creek	1998	43

*Cited from Sato et al. (2001).

except for those from three populations (Tsugaruishi River early-run in Japan, Namadae River in Korea, and Amur River in Russia), were from archives. The fish used in this study were captured when they returned to their natal rivers. Archived samples had been previously used for allozyme and/or mtDNA RFLP analyses (Kondzela et al. 1994, Wilmot et al. 1994, Winans et al. 1994, Seeb & Crane 1999b). Several populations were sampled in more than one year and we assumed that the genetic composition was temporally stable, as has been suggested by previous allozyme studies (Wilmot et al. 1994, Seeb & Crane 1999a,b). A possible genetic difference, between summer- and fall-run fish was examined for fish collected in the Yukon River. Early- and late-run populations from two Japanese rivers also were included in the analysis (Tokoro and Tsugaruishi Rivers), as in the previous study (Sato et al. 2001). Liver and blood samples were stored at -80°C and muscle samples were stored in 100% ethanol at room temperature until DNA extraction.

DNA extraction

DNA was isolated from the stored specimens following the phenol–chloroform method (Sambrook et al. 1989). Prior to extraction of DNA, the muscle samples were washed twice in 500 µl sodium–Tris–EDTA buffer (STE; 0.1 M NaCl, 10 mM Tris–HCl, and 1 mM EDTA, pH 8.0). The frozen liver samples were immediately homogenized in the same solution. About 50 µl of whole blood and homogenates of liver or muscle were added to 500 µl STE buffer containing 500 µg/ml proteinase K and 0.5% SDS, and incubated at 37°C overnight. DNA was extracted three times with a mixture of phenol (250 µl) and 24 : 1 chloroform : isoamylalcohol (250 µl), and then twice with 500 µl of the chloroform–isoamylalcohol alone. DNA in aqueous phase was recovered by ethanol precipitation, dried in air, and dissolved in Tris–EDTA buffer (TE; 10 mM Tris–HCl, 1 mM EDTA, pH 7.5).

PCR amplification and nucleotide sequence analysis

The control region of mtDNA was amplified by PCR as in the previous study (Sato et al. 2001). The PCR products were purified by the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) after confirmation of their sizes by gel-electrophoresis. Approximately 500 bp in the variable position of the 5′ end of the

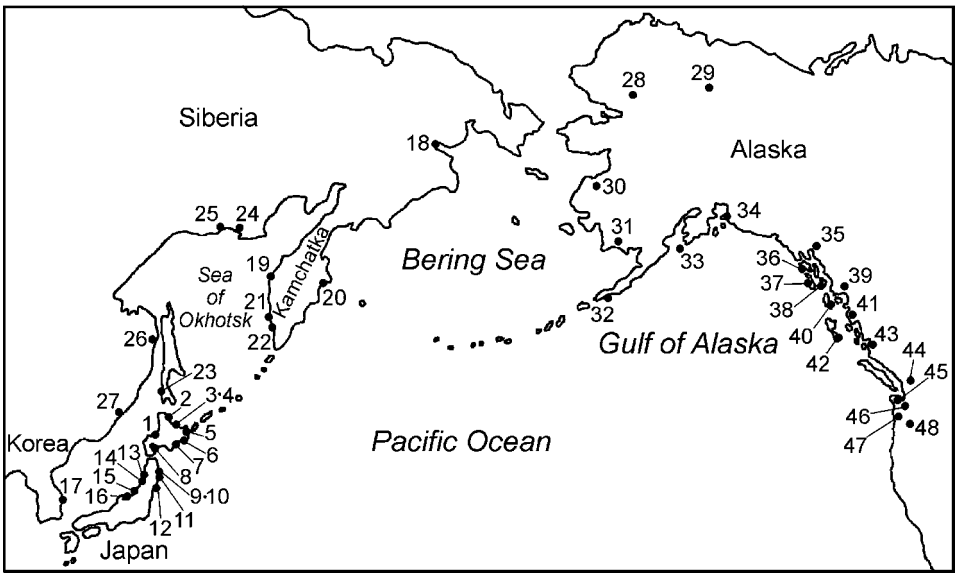

Figure 1. Geographical locations of sampling sites (see Table 1 for the site names).

mtDNA control region was sequenced with a Hitachi SQ-5500L DNA Sequencer (Hitachi, Tokyo) (Sato et al. 2001).

Nucleotide sequence data analysis

For data analysis, the nucleotide sequences of 537 previously examined individuals from 12 Japanese collections (11 populations) were also included. Thus, a total of 2,154 individuals from 48 Pacific Rim populations was analyzed in this study (Table 1 and Figure 1). The sequence data of the 5′ end of the mtDNA control region were aligned by GENETIX-WIN version 4.0.6 (Software Development Co. Ltd, Tokyo) to identify nucleotide variations, from which the haplotypes were defined. Haplotypes were connected into the parsimony network using two different programs: the Network version 3.111 program (Bandelt et al. 1999, available at the web site http://www.fluxus-engineering.com) and the TCS version 1.13 software (Posada & Crandall 2001, available at the web site http://zoology.byu.edu/crandall_lab/programs.htm).

Population genetic data analysis

Haplotype and nucleotide diversity within populations, and nucleotide divergence between populations were estimated according to Nei (1973) and Nei & Tajima (1981) using the DA program in REAP (McElroy et al. 1993). The heterogeneity of the haplotype frequencies within and between geographic regions was evaluated using the contingency χ^2 test (Roff & Bentzen 1989), with 10,000 Monte Carlo simulations by CHIRXC program (Zaykin & Pudovkin 1993). Populations were grouped by the neighbor-joining method (Saitou & Nei 1987) using pairwise nucleotide divergences. The topology obtained was tested for its stability by a consensus analysis using 1,000 replicates of the original population divergence matrix obtained by bootstrap resampling of individuals from each population. A neighbor-joining tree was constructed for each replicate with NEIGHBOR and the consensus tree was generated using CONSENSUS in PHYLIP version 3.5c software[1]. In order to assess the extent of genetic divergence at different levels of geographic hierarchy, the overall molecular variance was partitioned into components corresponding to the population divergence within and among regions by the analysis of molecular variance (AMOVA) model (Excoffier et al. 1992) using the Arequin version 2.000 program package[2].

[1] Felsenstein, J. 1993. PHYLIP (Phylogeny inference package), Version 3.5c. Department of Genetics, University of Washington, Seattle: Available at the web site http://evolution.genetics.washington.edu/phylip.html.

[2] Schneider, S., D. Roessli & L. Excoffier. 2000. Arlequin, Version 2.000 University of Geneva, Geneva: Available at the web site http://lgb.unige.ch/arlequin/.

Results

mtDNA control region haplotypes in chum salmon

Haplotype sequence variation. Sequence analysis of the 481 bp 5′ variable position of the mtDNA control region disclosed 20 variable sites in a total of 2,154 individuals from 48 populations, which defined a total of 30 haplotypes, which we designated as A-1 to C-5 (Table 2). The nucleotide sequence variations observed included one nucleotide insertion, one nucleotide deletion, and 18 nucleotide substitutions including 11 transitions and seven transversions (see Table 2). Designation of the 12 haplotypes reported in the previous study (Sato et al. 2001) were changed as follows: OKDL-1 to A-1, OKDL-2 to A-5, OKDL-3 to A-6, OKDL-4 to A-7, OKDL-5 to A-8, OKDL-6 to B-1, OKDL-7 to B-3, OKDL-8 to B-4, OKDL-9 to C-1, OKDL-10 to C-2, OKDL-11 to C-4, and OKDL-12 to C-5 (GenBank accession numbers AB039890–AB039901). The sequences of 18 newly identified mtDNA control region haplotypes were registered in the DDBJ/EMBL/GenBank with accession numbers AB091514–AB091531.

Haplotype genealogy. The observed haplotypes of chum salmon were grouped into three clades based on the nucleotide variation shown in Table 2, i.e., A-1 to A-8 in clade A, B-1 to B-17 in clade B, and C-1 to C-5 in clade C. The T to C transition at nucleotide 30 separated clade C from clade A, and a deletion at nucleotide 386 and C to A transversion at nucleotide 395 discriminated clade B from clades A and C, respectively, as shown in Table 2. Two different algorithms following Templeton et al. (1992) and Bandelt et al. (1999) created essentially the same parsimony network connecting the 30 control region haplotypes (data not shown). A single minimum spanning tree (Figure 2) was then produced

Table 2. Variable nucleotide positions in the 5′ half of mtDNA control region of chum salmon.

Haplotype	10	30	42	57	70	78	96	108	154	194	231	242	250	260	339	340	386	395	401	471
A-1	T	T	A	A	T	T	-	A	C	A	T	C	T	A	T	C	G	C	T	A
A-2	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
A-3	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
A-4	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·
A-5	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·
A-6	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·
A-7	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C
A-8	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·
B-1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	-	A	·	·
B-2	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	-	A	·	·
B-3	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	-	A	·	·
B-4	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	-	A	·	·
B-5	C	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	-	A	·	·
B-6	·	·	·	·	C	·	·	·	G	·	·	·	·	·	·	·	-	A	·	·
B-7	·	·	·	·	·	C	·	·	G	·	·	·	·	·	·	·	-	A	·	·
B-8	·	·	·	·	·	·	·	C	G	·	·	·	·	·	·	·	-	A	·	·
B-9	·	·	·	·	·	·	·	·	G	·	C	·	·	·	·	·	-	A	·	·
B-10	·	·	·	·	·	·	·	·	G	·	·	T	·	·	·	·	-	A	·	·
B-11	·	·	·	·	·	·	·	·	G	·	·	·	C	·	·	·	-	A	·	·
B-12	·	·	·	·	·	·	·	·	G	·	·	·	·	G	·	·	-	A	·	·
B-13	·	·	·	·	·	·	·	·	G	·	·	·	·	·	A	·	-	A	·	·
B-14	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	-	A	C	·
B-15	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	-	A	·	C
B-16	·	·	·	·	·	·	·	·	G	·	·	·	·	·	A	T	-	A	·	·
B-17	·	·	·	·	·	·	·	·	G	·	·	·	·	·	A	·	-	A	C	·
C-1	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
C-2	·	C	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
C-3	·	C	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
C-4	·	C	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·
C-5	·	C	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·

The nucleotide at each position is given for A-1. The hyphen represents the deletion and dot represents the same nucleotide at the same position as in the A-1.

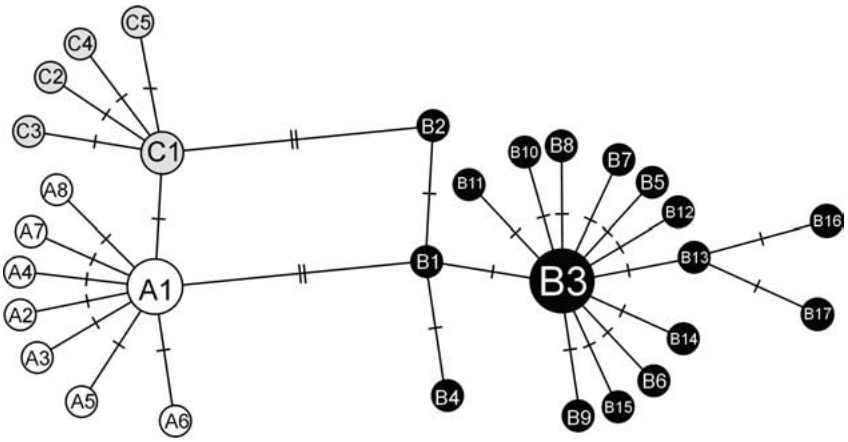

Figure 2. A single minimum spanning tree for the 30 mtDNA control region haplotypes (481 bp sequences) of chum salmon presented in Table 2. Circle sizes reflect haplotype abundances.

by assuming that transitions were more probable than transversions, that ambiguously positioned haplotypes were more likely to be descended from other haplotypes in their geographic neighborhood and from more abundant and interior haplotypes (Castelloe & Templeton 1994). A-1, B-3, and C-1 were focal haplotypes in their respective clades, from which rarer haplotypes were radiated, thus showing three star-like genealogies. However, the connection between these focal haplotypes remained ambiguous based on these data (Figure 2).

Haplotype distribution in the Pacific Rim populations

The distribution of 30 haplotypes among 48 populations of chum salmon is presented in Table 3. The frequency of haplotypes across the Pacific Rim was nonrandom, and mostly associated with regional structure.

Asian populations. The Japanese populations had the most haplotypes, 16 of 30, in the regions examined. All three haplotype clades were represented in every population except for the Tsugaruishi late-run (9) and the Otsuchi (11) populations both of which lacked clade B haplotypes (see Table 3 and Figure 1). The A-1 and C-1 haplotypes were common in all the populations of Japan; the B-3 was common in most Japanese populations. The frequency and composition of the haplotypes differed between early (10) and late (9) runs from the Tsugaruishi River on the Pacific coast of Honshu, whereas the Tokoro River early (4) and late (3) runs in Hokkaido showed similar haplotype distributions (Sato et al. 2001).

Thirteen haplotypes occurred in 10 Russian populations and four haplotypes occurred in a single Korean population (Table 3). All three haplotype clades were observed in the Namadae River (17) in Korea and the Avakumovka River (27) in Russia, both of which are on the Sea of Japan coast (see Table 3 and Figure 1). Nine other Russian populations contained clade B and C haplotypes, although the clade C haplotypes were less abundant than clade B haplotypes (Table 3). Haplotype B-3 was predominant in most of the Russian populations (Table 3).

North American populations. The number of haplotypes in North American populations was typically less than that of populations in Japan and Russia. The North American populations exhibited no clade A, 10 clade B, and one clade C haplotypes (Table 3). Clade C haplotypes were rare (<8%) and occurred in only two populations, one from the Alaska Peninsula (Belkofski River, 32) and the other from Kodiak Island (Kizhuyak River, 33), both areas of Southcentral Alaska (see Table 3 and Figure 1). Among the observed clade B haplotypes, B-3 and B-13 were common in most of the North American populations, although the latter was less frequent than the former. Four populations in western Alaska, Salmon River (28), fall-run (Sheenjak River, 29) and summer-run (Andreafsky River, 30) of the Yukon River, and Togiak River (31), lacked the B-13 haplotype and were fixed or nearly fixed for the

Table 3. Distribution of mtDNA control region haplotypes among 48 populations of chum salmon in the Pacific Rim.

Population no.	Number of individuals with haplotype																												
	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	C1	C2	C3	C4	C5
Japan																														
Hokkaido Island																														
1	22	0	0	0	1	0	0	0	0	0	14	2	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	2
2	30	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0
3	26	0	0	0	0	0	1	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	1
4	21	0	0	0	0	1	0	0	1	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	1
5	12	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0
6	23	1	4	0	0	0	0	0	1	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	1	0	1
7	18	0	0	0	0	2	0	4	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	1	0	1	0
8	24	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0
Honshu Island																														
9	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	0	0	0	0
10	20	0	0	0	0	4	0	1	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0
11	26	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0	0
12	24	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0	0
13	19	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	1
14	26	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	0	0	0
15	29	0	0	2	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	1
16	37	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0
Korea																														
17	36	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1
Russia																														
Anadyr																														
18	0	0	0	0	0	0	0	0	0	0	33	0	1	0	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0
Kamchatka Peninsula																														
19	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0	1	0	0	0	0	0	0	0	0	8	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	31	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0	1	0	0	1	0	0	0	0	0	6	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0	2	0	0	1	0	0	0	0	0	3	0	0	0	0
Sakhalin Island																														
23	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	6	16	0	0	0	0	0	0	0	0	7	0	0	0	1
Magadan																														
24	0	0	0	0	0	0	0	0	1	0	20	0	0	1	0	0	3	0	0	0	0	0	0	0	0	7	0	0	0	1
25	0	0	0	0	0	0	0	0	0	4	31	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0

Table 3. (*Continued*)

Population no.	Number of individuals with haplotype																												
	A1	A2	A3	A4	A5	A6	A7	A8	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	C1	C2	C3	C4	C5
Nikolaevsk-na-Amure																														
26	0	0	0	0	0	0	0	0	2	0	45	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	0
Primorye																														
27	7	0	0	0	0	0	0	0	0	0	9	0	0	0	0	1	0	0	0	0	0	0	0	0	0	6	0	0	0	7
North America																														
Northwest Alaska																														
28	0	0	0	0	0	0	0	0	0	0	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0	48	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Alaska Peninsula																														
32	0	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0	0	0	5	0	0	0	0	4	0	0	0	0
Southcentral Alaska																														
33	0	0	0	0	0	0	0	0	0	0	36	0	0	0	0	0	1	0	0	0	6	0	0	0	0	1	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	35	0	0	0	0	0	0	0	2	0	6	0	0	0	2	0	0	0	0	0
Southeast Alaska																														
35	0	0	0	0	0	0	0	0	0	0	39	0	0	0	0	0	1	0	5	0	5	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	40	0	0	0	0	0	1	0	1	0	7	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	2	0	0	0	13	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	40	0	0	0	0	0	4	0	0	0	1	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	5	0	0	0	12	0	0	0	0	0	0	0	0	0
British Columbia																														
41	0	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0	1	0	0	0	15	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	32	0	0	0	0	0	0	0	1	0	17	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0
Washington																														
44	0	0	0	0	0	0	0	0	0	0	39	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	41	0	0	0	0	0	0	3	0	0	5	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0	0	0	0	0	0	3	0	0	2	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	23	0	0	0	0	0	1	0	0	0	17	8	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	23	0	6	0	0	0	0	0	0	0	12	2	0	0	0	0	0	0	0	0
Total	425	1	4	2	1	10	1	5	5	4	1206	3	7	1	1	7	42	3	9	2	143	10	1	2	3	236	2	1	1	16

B-3 haplotype (Table 3 and Figure 1). Other North American populations usually included one or two clade B haplotypes in addition to the B-3 and B-13 haplotypes.

Inter-regional haplotype distribution. Among the observed haplotypes, 22 were region-specific, including seven clade A (A-2 to A-8), one clade B (B-4) and two clade C (C-3 and C-4) of 16 haplotypes in Japan; seven clade B (B-10, B-11, B-13 to B-17) of 11 haplotypes in North America; and five clade B (B-2, B-6 to B-8, and B-12) of 13 haplotypes in Russia (Table 3). The B-3 and C-1 haplotypes occurred in all three geographic regions, although the haplotype C-1 was rare and its occurrence was limited in the North American populations. The A-1, B-1, C-2, and C-5 haplotypes occurred in both the Japanese and Russian populations, and the B-5 and B-9 were shared in the Russian and North American populations. Therefore, it is reasonable to conclude that clade A and C haplotypes characterize the Asian populations, and clade B haplotypes predominate in the North American populations.

Population genetic analysis

Haplotype diversity was highest in the populations of Japan (0.63 ± 0.01), followed by those of Russia (0.43 ± 0.03) and North America (0.34 ± 0.02), whereas nucleotide diversity was similar in the Japanese (0.0028) and Russian populations (0.0025), but lower in the North American populations (Table 4). These findings suggest greater genetic variation in the populations of Japan than those of Russia and North America.

Population clustering. The populations examined were clustered using the neighbor-joining method (Figure 3). The population consensus tree clearly separated Japan/Korea from the other geographic groups with high bootstrap support (99%), although one Russian population (Avakumovka) on the Sea of Japan coast was included in the Japan/Korea cluster. Interestingly, western Alaskan populations were separated from the other North American groups with more than 50% of the bootstrap replicates, but included two Russian populations (the Kamchatka River in eastern Kamchatka Peninsula and the Kalininka River in Sakhalin Island) in the same cluster. Other Russian populations formed a separate cluster with more than 95% bootstrap support. Thus, four major population clusters of Japan/Korea, Russia, Northwest Alaska, and the rest of the North American groups were apparent on the consensus tree.

Heterogeneity in the haplotype distribution. The contingency χ^2 test showed highly significant heterogeneity ($p < 0.001$) in the haplotype frequencies for the entire set of populations (Table 5). Significant regional heterogeneity ($p < 0.001$) was also observed between each set of populations from Japan and Russia, Japan and North America, and Russia and North America, respectively. Furthermore, significant heterogeneity was observed among North American populations ($p < 0.005$), which suggested five subregional North American groups: western Alaska, the Alaska Peninsula/Southcentral Alaska, Southeast Alaska, British Columbia, and Washington (Table 5). A similar level of heterogeneity ($p < 0.005$) was also apparent among Hokkaido, the Pacific coast of Honshu, and the Sea of Japan coast in the Japanese populations (Table 5, Sato et al. 2001). No significant heterogeneity was observed among the populations in the Yukon summer- and fall-runs in western Alaska or the late- and early-runs in the Tokoro River in Hokkaido (Sato et al. 2001), whereas significant heterogeneity was observed between the Tsugaruishi late- and early-runs on the Pacific coast of Honshu ($p < 0.01$).

Geographic hierarchy in the Pacific Rim populations. Using AMOVA to partition of the molecular variance AMOVAs (Table 6) revealed the following population structure in chum salmon: (i) very strong geographic structuring among Japan, Russia, and North America (56.2% of the total variance, Analysis I), as compared with the average extent of structuring among populations within each geographic group (4.3% of the total variance); (ii) weak to moderate structuring among western Alaska, Alaska Peninsula/Southcentral Alaska, Southeast Alaska, British Columbia, and Washington (4.9% of the variance, Analysis IV); (iii) similar level of intra-regional structuring among Hokkaido, the Pacific coast of Honshu and the Sea of Japan coast of Honshu in Japan (7.3% of the variance, Analysis II) as described previously (Sato et al. 2001); and (iv) unclear geographic structuring among six regional groups (Tables 1 and 3) (18.2% of the variance, $p = 0.075$, Analysis III) and very weak structuring among local populations within groups in Russia (3.0% of the variance). The latter is likely associated

Table 4. Haplotype diversity (h, ±SD) and nucleotide diversity (π, in parentheses) within 48 populations calculated from mtDNA haplotype frequencies.

Population	h (π)	Population	h (π)	Population	h (π)
Japan	0.63 ± 0.01 (0.0028)	*Russia*	0.43 ± 0.03 (0.0025)	Sawmill Cr.	0.38 ± 0.08 (0.00085)
Chitose	0.71 ± 0.04 (0.0038)	Anadyr	0.38 ± 0.08 (0.0029)	Long Bay	0.32 ± 0.08 (0.00069)
Tokushibetsu	0.58 ± 0.05 (0.0030)	Hairusova	0.36 ± 0.08 (0.0028)	Whale Bay	0.46 ± 0.06 (0.0010)
Tokoro	0.60 ± 0.07 (0.0028)	Kamchatka	0.06 ± 0.06 (0.00013)	Port Beauclere	0.21 ± 0.08 (0.00044)
Tokoro-E	0.69 ± 0.04 (0.0037)	Vorovskaya	0.31 ± 0.08 (0.0021)	Fish Cr.	0.16 ± 0.07 (0.00040)
Nishibetsu	0.67 ± 0.03 (0.0040)	Kol	0.25 ± 0.08 (0.0014)	Disappearance Cr.	0.51 ± 0.06 (0.0012)
Kushiro	0.72 ± 0.05 (0.0032)	Kalininka	0.63 ± 0.04 (0.0016)	Ecstall	0.48 ± 0.05 (0.0010)
Tokachi	0.75 ± 0.04 (0.0039)	Ola	0.59 ± 0.08 (0.0038)	Bag Harbor	0.48 ± 0.05 (0.0010)
Yurappu	0.57 ± 0.06 (0.0024)	Magadan	0.33 ± 0.09 (0.0019)	Nekite Channel	0.38 ± 0.08 (0.00079)
Tsugaruishi	0.50 ± 0.03 (0.0010)	Amur	0.19 ± 0.07 (0.00065)	Nooksack	0.29 ± 0.07 (0.00060)
Tsugaruishi-E	0.70 ± 0.04 (0.0030)	Avakumovka	0.79 ± 0.03 (0.0048)	Quilcene Bay	0.29 ± 0.08 (0.00063)
Otsuchi	0.54 ± 0.03 (0.0012)			Blackjack Cr.	0.19 ± 0.07 (0.00055)
Koizumi	0.55 ± 0.03 (0.0014)	*N. America*	0.34 ± 0.02 (0.00083)	Satsop	0.65 ± 0.04 (0.0016)
Kawabukuro	0.56 ± 0.09 (0.0026)	Salmon	0.00 ± 0.00 (0.0000)	Hamilton Cr.	0.63 ± 0.05 (0.0016)
Gakko	0.57 ± 0.05 (0.0022)	Sheenjek	0.00 ± 0.00 (0.0000)		
Uono	0.60 ± 0.06 (0.0027)	Andreafsky	0.00 ± 0.00 (0.0000)	Total	0.63 ± 0.01 (0.0037)
Jintsu	0.39 ± 0.07 (0.0012)	Togiak	0.04 ± 0.04 (0.000085)		
		Belkofski	0.34 ± 0.08 (0.0018)		
Korea		Kizhuyak	0.32 ± 0.08 (0.00097)		
Namadae	0.37 ± 0.08 (0.0019)	Olsen Cr.	0.38 ± 0.08 (0.00099)		

with an insufficient number of populations in the study, i.e., a single population in four of the locales.

Discussion

This mtDNA analysis resolved 20 variable positions in the approximately 500 bp nucleotide sequences at the 5′ end of the control region (Table 2), which define a total of 30 haplotypes among more than 2,100 individuals from 48 populations of chum salmon in the Pacific Rim (Table 3). Analysis of the variation among populations demonstrated; (i) substantial genetic divergence among three geographic groups of chum salmon, i.e., Japan, Russia, and North America; (ii) greatest genetic variation between Japanese and North American populations; and (iii) weak to moderate genetic isolation within Japanese and North American populations.

Figure 3. Unrooted neighbor-joining tree showing net nucleotide divergence among 48 chum salmon populations. The scale corresponds to 0.1% of nucleotide divergence. In the inset, the topology of the consensus tree (not scaled) is shown with nodal values for bootstrap support over 50% of the 1,000 replicated trees. The number in parenthesis indicates the geographical location of each population shown in Figure 1.

Table 5. Results of homogeneity test for pairwise geographic regions with indicated number of chum salmon populations in parenthesis. The probability of homogeneity for pairwise geographic regions was given below diagonal, which was calculated using contingency χ^2 test with 10,000 Monte Carlo simulations (Roff & Bentzen 1989). The probability of homogeneity within regions was given on diagonal.

Region	Japan		Russia	North America				
	HOK	HON	RUS	NWA	AP/SCLA	SEA	BCL	WSG
Japan								
Hokkaido (8)	<0.001							
Honshu (8)	<0.001	<0.001						
Russia								
Russia (10)	<0.001	<0.001	<0.001					
North America								
Northwest Alaska (4)	<0.001	<0.001	<0.001	1				
Alaska Peninsula/ Southcentral Alaska (3)	<0.001	<0.001	<0.001	<0.001	0.069			
Southeast Alaska (6)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
British Columbia (3)	<0.001	<0.001	<0.001	<0.001	<0.005	<0.001	0.75	
Washington (5)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Our nucleotide sequence analysis detected similar or higher levels of variation than that found at the 3′ end of the control region in other *Oncorhynchus* species, including *O. mykiss*, *O. kisutch*, and *O. tshawytscha* (Nielsen et al. 1994). Such inter-specific comparisons must be interpreted with care, because the extent of sequence variation in the control region is site-specific and species-specific in fish (Meyer 1993). However, the

Table 6. Results of the hierarchical analyses of molecular variance for chum salmon.

Variance component	%	P	Φ
Analysis I			
Among three regional groups (Japan, Russia, and North America)	56.2	<0.001	0.56
Among populations within groups	4.3	<0.001	0.098
Within populations	39.5	<0.001	0.60
Analysis II			
Among three regional groups in Japan	7.3	<0.001	0.073
Among populations within groups	1.5	<0.05	0.017
Within populations	91.2	<0.001	0.088
Analysis III			
Among six regional groups in Russia	18.2	0.075	0.18
Among populations within groups	3.0	<0.001	0.03
Within populations	78.8	<0.001	0.21
Analysis IV			
Among five regional groups in North America	4.9	<0.005	0.049
Among populations within groups	3.7	<0.001	0.038
Within populations	91.4	<0.001	0.085

The percentage of variance (%), probability estimated from permutation (P), and the F-statistics (Φ) are given at hierarchical level (Exoffier et al. 1992).

variation found herein was higher than that detected in previous RFLP analyses of chum salmon mtDNA in terms of the number of the observed haplotypes (Cronin et al. 1993, Park et al. 1993, Seeb & Crane 1999b, Churikov et al. 2001). These observations indicate the potential of mtDNA sequence analysis to estimate the genetic variation and to examine the population structure of chum salmon.

The thirty haplotypes observed were genealogically connected in the three clades, although their relationships are ambiguous (Figure 2). The A-1, B-3, and C-1 haplotypes are presumably ancestral to other haplotypes within clades A, B, and C, respectively, given their abundance and centrality in each genealogy. The three star-like genealogies suggest that most of the rarer haplotypes radiating from the central ones may have evolved after colonization of chum salmon in each of the three geographic regions. This is supported by the observation that the radiated haplotypes include most of the region-specific haplotypes. The Japanese populations have some features not found in other geographic groups: the largest number of haplotypes including the region-specific ones, the greatest haplotype diversity, and the presence of all three haplotype clades. These results suggest that the Japanese populations have longer histories than Russian and North American populations.

The neighbor-joining tree, contingency χ^2 test, and AMOVA revealed clear geographic structuring in the Pacific Rim chum salmon populations, with distinct genetic divergence among Japan, Russia, and North America. Genetic divergence among the three regional groups of chum salmon was also observed in previous studies using variation of allozyme loci (Okazaki 1983, Wilmot et al. 1998, Seeb & Crane 1999b), mtDNA RFLPs (Seeb & Crane 1999a), and minisatellite DNA (Taylor et al. 1994). Furthermore, several studies have also inferred geographic structuring within regions (Kondzela et al. 1994, Phelps et al. 1994, Wilmot et al. 1994, 1998, Seeb & Crane 1999b). In this study, subregional structure was observed between populations within Japan and North America, although the extent of structure within regions was weak to moderate as compared with inter-regional structure (Table 6). In Japan, low or restricted gene flow between Hokkaido and Honshu was implied by higher F_{ST} estimates between Hokkaido and Honshu populations than within Hokkaido or Honshu populations (Sato et al. 2001). Low gene flow between the two regions in Japan may result from differences in the route of spawning migration, run timing, and distance between populations, in addition to possible geological or historical factors (Sato et al. 2001). Over the past few decades, however, contemporary forces such as extensive human-mediated transplantation of stocks may have eroded an even stronger previously existing structure. The Hokkaido and Honshu populations have undergone extensive hatchery operations and transplantation of eggs and fry from one river population to another in the history of commercial salmon production (Kijima & Fujio 1982). In this study, we attempted to minimize such a contemporary influence by sampling late-run fish, particularly in Honshu, because introduced Hokkaido populations migrate earlier than native Honshu populations (Salo 1991).

The population structure within North America, i.e., western Alaska, Alaska Peninsula/Southcentral Alaska, Southeast Alaska, British Columbia, and Washington, is also intriguing. Although no study exists that encompasses all of North America, some of the regional structure was observed in previous allozyme studies (Kondzela et al. 1994, Wilmot et al. 1998, Seeb & Crane 1999b). Contingency χ^2 tests of our mtDNA data indicate low gene flow among these

five regions, particularly between western Alaska and other North American locales (Table 5). Although all the factors involved in the structure observed within North American salmon have not been determined, the glacial history of this region has likely had an influence (Utter et al. 1980, Gharrett et al. 1987, Varnavskaya & Beacham 1992, Cronin et al. 1993, Varnavskaya et al. 1994, Bickham et al. 1995, Seeb & Crane 1999a). Two refugia, i.e., Beringia and Cascadia, have been suggested as a Pleistocene cradle for North American salmon species including chum salmon; whereas salmonid populations of western Alaska and Russia were probably recolonized from Beringia, the populations of the Gulf of Alaska, British Columbia, and Washington were recolonized from Cascadia (Seeb & Crane 1999a). The geographic grouping of North American chum salmon populations in this mtDNA analysis may reflect the glacial history, although this inference needs confirmation by further studies including additional collections, particularly from Russia, and analytical methods that estimate population history, such as nested cladistic analysis (Templeton et al. 1992).

The distinct genetic population structure that we observed in Pacific Rim chum salmon revealed herein also suggests that mtDNA sequence analysis may provide good regional estimates of stocks in mixed-stock ocean aggregates. Additional populations from Russia and North America are needed to establish the mtDNA baseline for accurate stock identification. As mentioned above, our mtDNA sequence data may be useful for analyzing the evolutionary mechanisms that shaped the current geographic distribution of chum salmon in the Pacific Rim. Such studies are now ongoing in our laboratories.

Acknowledgements

We would like to thank Dmitri Churikov and Anthony Gharrett, Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks, for valuable advice on statistical analysis. We also would like to thank the Tsugaruishi fisherman's cooperative association for their help in sampling. This study was supported in part by Grants-in-Aid from the Fisheries Agency, Northern Advancement Center for Science and Technology, and the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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