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Moongeun Yoon[1], Shunpei Sato[2], James E. Seeb[3], Richard L. Wilmot[4], Shigehiko Urawa[2], Akihisa Urano[5], Syuiti Abe[1]

[1]Division of Marine Biosciences, Graduate School of Fisheries Sciences, Hokkaido University, Japan
[2]Genetics Section, National Salmon Resources Center, Japan
[3]Alaska Department of Fish and Game, U.S.A.
[4] Auke Bay Laboratory, Alaska Fisheries Science Center, NOAA, U.S.A.
[5]Division of Biological Science, Graduate School of Sciences, Hokkaido University, Japan

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Genetic variation among chum salmon populations in the Pacific Rim
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Moongeun Yoon[1], Shunpei Sato[2], James E. Seeb[3], Richard L. Wilmot[4], Shigehiko Urawa[2], Akihisa Urano[5], Syuiti Abe[1]

[1]Division of Marine Biosciences, Graduate School of Fisheries Sciences, Hokkaido University, Japan
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[3]Alaska Department of Fish and Game, U.S.A.
[4] Auke Bay Laboratory, Alaska Fisheries Science Center, NOAA, U.S.A.
[5]Division of Biological Science, Graduate School of Sciences, Hokkaido University, Japan

ABSTRACT

We performed a combined analysis of mitochondrial (mt) DNA control region sequence and four polymorphic microsatellite (ms) DNA loci to estimate the genetic differentiation and genetic structure among chum salmon populations in the Pacific Rim. Nucleotide sequence analysis of the mtDNA control region revealed 22 variable nucleotide sites, which defined a total of 32 haplotypes of three clades (A, B and C), in more than 4,200 individuals representing 96 populations including newly collected 20 populations from North America. The occurrence of haplotypes was in keeping with our previous observations, in that clade A and C haplotypes characterized Asian populations and clade B haplotypes distinguished North American populations. The analysis of molecular variance and pairwise population F_{ST} estimation further advocate our previous findings, which include the highest genetic variation in the Japanese populations, strong structuring among Japanese, Russian and North American populations, and a substantial geographic structuring among local populations within regions. Similar population genetic analyses with msDNA using more than 1,100 individuals from 24 populations of Japan (14) and North America (10) also disclosed a greater genetic diversity in the populations of Japan than North America and a distinct genetic differentiation between and within geographical groups of populations. The observed congruence in the results from mtDNA and msDNA analyses suggests that the battery of two DNA markers will become useful for construction of the baseline for better genetic stock identification of mixed chum salmon populations in high seas.

INTRODUCTION

Chum salmon have received considerable attention due to their wide geographic distribution in the Pacific Rim (Salo 1991) and high commercial importance. Stock identification of chum salmon in mixed aggregations in high seas is therefore a fundamental international issue. Recently developed molecular techniques are expected to provide a powerful means with an increased accuracy and resolution to reveal genetic variation in salmon populations. However, sufficient information has not yet been accumulated to conclude which DNA markers improve discrimination among populations and whether any variation in the utility of different markers exists at different geographic scale of analysis.

Recently, Sato et al. (2001; 2004a) detected greater amount of variation in the mtDNA control region by nucleotide sequence analysis than the variation observed by a previous RFLP

analysis (Park et al. 1993). The mtDNA sequence analysis using more than 3,000 fish representing 76 chum salmon populations in the Pacific Rim revealed a clear geographic structuring among Japan, Russia and North America and among local populations within regions (Yoon et al. 2004). These findings suggest the possibility of mtDNA sequence variation to become a competent marker for genetic stock identification (GSI) of chum salmon (Sato et al. 2004b, Yoon et al. 2004), if the comprehensive baseline data could be established without gaps in collection sites along the Pacific Rim.

Microsatellite DNA with tandem repeats of 2 to 4 base short motifs is a class of highly polymorphic nuclear DNA markers that are suitable for studies on the level of intraspecific genetic diversity and population structure (Bruford & Wayne 1993). A variety of polymorphic msDNA loci have so far been developed for the population analysis of Pacific salmon, with a hope to utilize them as a tool of GSI (Beacham and Candy 2005, Habicht et al. 2005).

In this study, we recruited 20 additional chum salmon populations from North America in the mtDNA sequence analysis to see if more sequence variation could be detected to improve estimation of genetic diversity and population structure in the Pacfic Rim. Also, msDNA analysis was performed with four polymorphic loci (Abe et al. 2002) using Japanese and North American populations to compare the genetic features obtained from mtDNA analysis.

MATERIALS AND METHODS

Sampling profile

Liver, fins, or muscle samples of chum salmon were collected from 976 individuals from 20 North America populations for the mtDNA analysis (Table 1 and Figure 1). For the msDNA analysis, 1,186 individuals of 24 populations from Japan (14 populations) and North America (10 populations of Northwest, Southcentral Alaska, and Alaska Peninsula) were used, all of which were the same samples as used for the mtDNA analysis. Liver samples were stored at –80°C, and fins and muscle samples were kept in ethanol at room temperature until DNA extraction.

Mitochondrial and microsatelliteDNA analyses

DNA was isolated with conventional phenol-chloroform method (Sambrook et al. 1989) from the stored specimens as in our previous studies (Sato et al. 2001, 2004). Approximately 500 bp in the variable position of the 5'end of the mtDNA control region was sequenced with an ABI PRISM 3130 xl genetic analyzer (Applied Biosystems) (Sato et al. 2001), in addition to the analysis using the newly developed DNA microarray hybridization method (Moriya et al. 2004).

Four polymorphic msDNA loci (OKM4, OKM5, OKM7 and OKM8) (see also Abe et al. 2002) were examined by the polymerase chain reaction (PCR) amplification, in which one primer of each primer set was labeled with the fluorescent dyes: FAM, NED, NIC or PET. The optimization of PCR conditions determined for chum salmon were one min pre-denature at 95℃, followed by 30 cycles of denaturation at 95℃ for 15 sec, annealing at 57℃ (except for OKM5 at 55℃) for 15 sec, and extension at 72℃ for 60 sec. The PCR products were analyzed on an ABI PRISM 3130XL Genetic analyzer (Applied Biosystems).

Population genetic data analysis

Departure from Hardy-Weinberg equilibrium (HWE) and Genotype frequency at each locus were assessed in each population using Genepop version 3.4 program packages. Gene diversity according to Nei (1987) for the msDNA and pairwise population F_{ST} estimation with mtDNA were performed using the Arlequin version 2.000 program package (Schneider et al.

2000). A neighbor-joining tree inferred from msDNA data was constructed with NEIGHBOR and the consensus tree was generated using CONSENSUS in PHYLIP version 3.5c software (Felsenstein 1993). Other population genetic parameters such haplotype and nucleotide diversities with mtDNA also were estimated using the Arlequin program. 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 model (AMOVA; Excoffier et al. 1992) using the above Arlequin program for both mtDNA and msDNA analyses.

RESULTS

Mitochondrial DNA analysis

Estimation of the 481 bp sequence in the 5' variable portion of chum salmon mtDNA control region disclosed 22 variable sites in 4,243 individuals from 96 populations including previously analyzed 76 populations (1-76) (Yoon et al. 2004) and additional 20 North American populations (77-96) (Table 1 and Figure 1), which defined a total of 32 haplotypes including the previously defined 30 haplotypes (Sato et al. 2004) and newly identified two haplotypes of clade B (Table 2) from WHN Hatchery (82, 83) and Marten River (85) in the 20 additional populations (Table 3).

The number of haplotypes in North American populations was apparently less than that of populations in Japan and Russia even after addition of the 20 additional populations (Yoon et al. 2004; also see Table 3). The North American populations exhibited no clade A haplotypes, and one clade C haplotype occurred in only Toklet River (77) (see Table 3 and Figure 1).

Two novel haplotypes of clade B were designated to B-11 and B-16 by nucleotide variation (Table 2) and on a parsimony network (Figure 2). Therefore, the 7 haplotypes reported in the previous study (Sato et al. 2004) were changed as follow: B-11 to B-12, B-12 to B-13, B-13 to B-14, B-14 to B-15, B-15 to B-17, B-16 to B-18 and B-17 to B-19, respectively. Nucleotide divergence and the number of net nucleotide substitution per sites between clade A and C was lower than those between A and C and between B and C, suggesting a closer genetic kinship between A and C than between B and C and between A and B lineages (Table 4).

The observed haplotype distribution in the present 20 populations from North America was mostly the same as those found in our previous observations (Sato et al. 2004) and further advocated a geographic association of haplotypes, in that clade A and C haplotypes characterized Asia and Russian populations and clade C haplotypes distinguish Alaskan population.

Haplotype diversity was highest in the populations of Japan (0.607±0.001), followed by those of Russia (0.359±0.001) and North America (0.174±0.001), whereas nucleotide diversity was nearly similar in the Japanese (0.0021) and Russian populations (0.0017), but lower in the North American populations (Table 5). These findings suggest a greater genetic variation in the populations of Japan than those of Russia and North America.

As shown in Table 6, pairwise F_{ST} estimates were greater between Japan and North America (0.667 to 0.905) than between Japan and Russia (0.013 to 0.883) or between Russia and North America (0.000 to 0.867). The AMOVAs (Table 7) revealed the following population structure in the Pacific Rim chum salmon populations; very strong geographic structuring among Japan, Russia, and North America (68.7% of the total variance, $p<0.001$, Analysis I), as compared with the average extent of structuring among populations within each geographic group (5.0% of the total variance), similar level of population structuring among three regional groups in Japan (7.8% of the variance, $p<0.001$, Analysis II), among five regional groups in North America (2.5% of the variance, $p<0.001$, Analysis IV), and among six regional groups of Russia (29.0% of the variance, $p<0.001$, Analysis III). These results suggest more definite

geographic structuring among regional groups than our previous study (Yoon et al. 2004).

Microsatellite DNA analysis

The number of alleles per locus across all populations was highest at the OKM8 locus with 16 in the Chitose River and lowest at the OKM7 with 1 in the Upper Nushagak River and Donjek River. The gene diversity per population was ranged from 0 at the OKM7 in two North American populations to 0.887 at the OKM4 in one Japanese population. Also, the expected heterozygosity within Japanese populations (0.262-0.889) was higher than North American populations (0-0.682) (Table 8). These finding suggest greater genetic variation in the populations of Japan than North America

A total number of 79 alleles were observed across the four loci, which ranged from 16 alleles at the OKM5 to 29 alleles at the OKM8 over all populations, and the observed heterozygosity for all populations ranged from 0.277 at the OKM7 to 0.681 at the OKM8 (Table 8), reflecting the large difference in number of alleles and between these loci.

A deviation from HWE assessed by Markov chain procedure (P<0.01) was observed in the Tokoro River (early run), Nishibetsu River, Gakko River, Kwethluk River and South fork kuskokwin River at the OKM8 locus. The deviation was also observed in the Tokachi River and Tanana River at the OKM4, and the Uono River, South fork kuskokwin River and Pelly River at the OKM5. Significant disequilibrium was found in the Chiginagak River at all of the examined microsatellite loci (Table 8).

The populations examined were clustered using the neighbor-joining method based on Nei's genetic distance (Figure 3). The population consensus tree clearly separated all Japanese populations from the 10 North American populations, although the Chiginagak River in the Alaska Peninsula was clearly separated from the other North America populations with high bootstrap support (100%).

AMOVAs (Table 9) indicated that most variation occurred within populations with the high variability (more than 87%) of all the microsatellite loci used. Even though msDNA failed to show genetic differentiation among regional group in Japan (P>0.05), there was significant structuring among Japan and North America (P<0.001) and among regional groups in North America, i.e. Northwest, Southcentral Alaska, and Alaska Peninsula.

DISCUSSION

In the present study, recruitment of 20 North American populations of chum salmon for mtDNA sequence analysis resolved 22 variable positions in about 500 bp nucleotide sequence at the 5' end of the control region (Table 2), which defined a total of 32 haplotypes in more than 4,200 individuals representing 96 populations of chum salmon in the Pacific Rim. Two novel haplotypes were both clade B lineage, but the haplotype and nucleotide diversities in North America remained the lowest among three regions. Geographic association of haplotypes and population genetic features with the present mtDNA data also were in keeping with our previous observations (Sato et al. 2004, Yoon et al. 2004).

The AMOVAs and pairwise F_{ST} estimates with the present mtDNA data revealed clear geographic structuring in the Pacific Rim chum salmon populations among and within Japan, Russia, and North America. Incorporation of 20 North American populations thus provided an increased resolution in the geographic differentiation among three regional groups of populations (68.7%, P<0.001, Table 7) as compared with the previous estimation (56.8%, P<0.001) by Yoon et al. (2004). These results suggest that the detailed sampling design, particularly with recruitment of more North American and Russian populations, is essential to improvement of genetic resolution for geographic structure of Pacific Rim chum salmon populations.

The present msDNA analysis revealed substantial genetic diversity in the examined populations, providing fairly good discrimination among regional populations of Japan and North America. In North America, three regional groups were more clearly structured (8.27%, P<0.001 and Φ=0.08) than did by mtDNA (2.1%, P<0.001 and Φ=0.02), as shown by AMOVA (Tables 7 and 9). Estimates of the extent of population differentiation by msDNA and mtDNA are mostly congruent, although F_{ST} values by msDNA (data not shown) seems generally low compared with those by mtDNA (see Abe et al. 2002). It is unknown, however, for the reason of the failure to demonstrate the geographic structure in Japan by AMOVA (Table 9) with four msDNA loci. Increased number of polymorphic loci may be worth attempt to confirm the present results.

Based on the high degree of genetic divergence among and within geographical groups of populations revealed by the present mtDNA and msDNA analyses, a battery of these two DNA markers seems to provide better resolution in the divergence estimation than that expected from the previous allozyme and other DNA analyses. Both DNA markers here concerned should therefore be applied in mixed samples of salmon aggregations in high seas, to evaluate the discriminative potential in GSI of chum salmon. In parallel with such an attempt, continuous efforts must be made to establish the baseline data with both markers, which is essential to substantiation of marker utility in mixed-stock identification of Pacific salmon.

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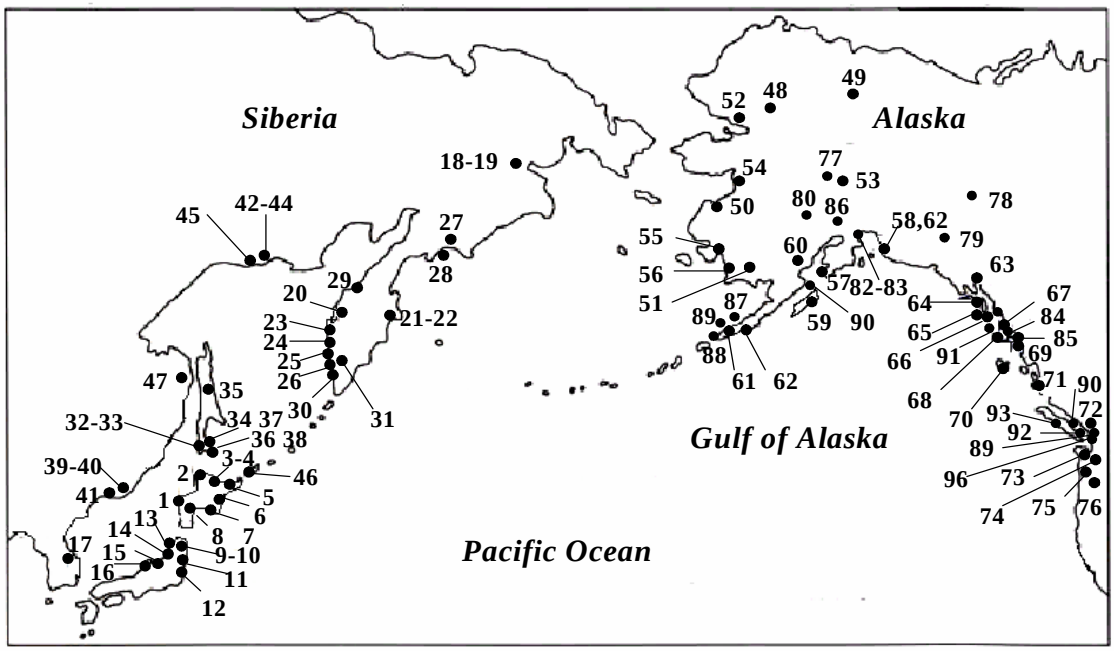

Fig. 1. Geographical position of sampling site (for the site names, see Yoon et al. 2004 for 1 to 76 and Table 1 for 77 to 96).

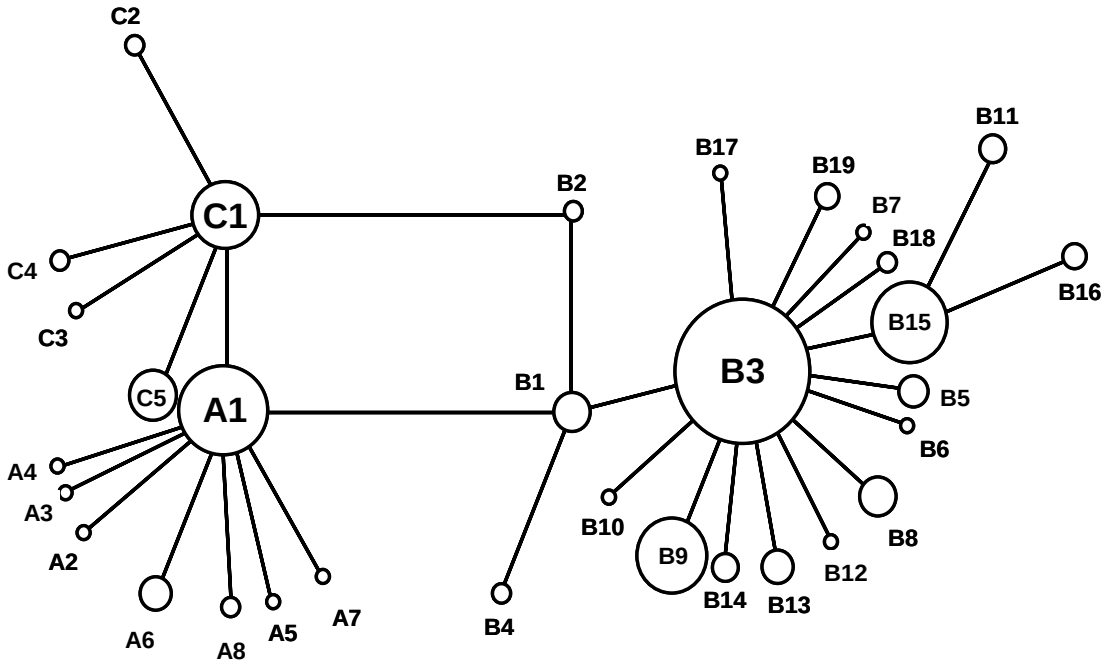

Fig. 2. A single minimum spanning tree for the 32 mtDNA control region haplotypes (481 bp) of chum salmon presented in Table 2. Circle sizes reflect haplotype abundance.

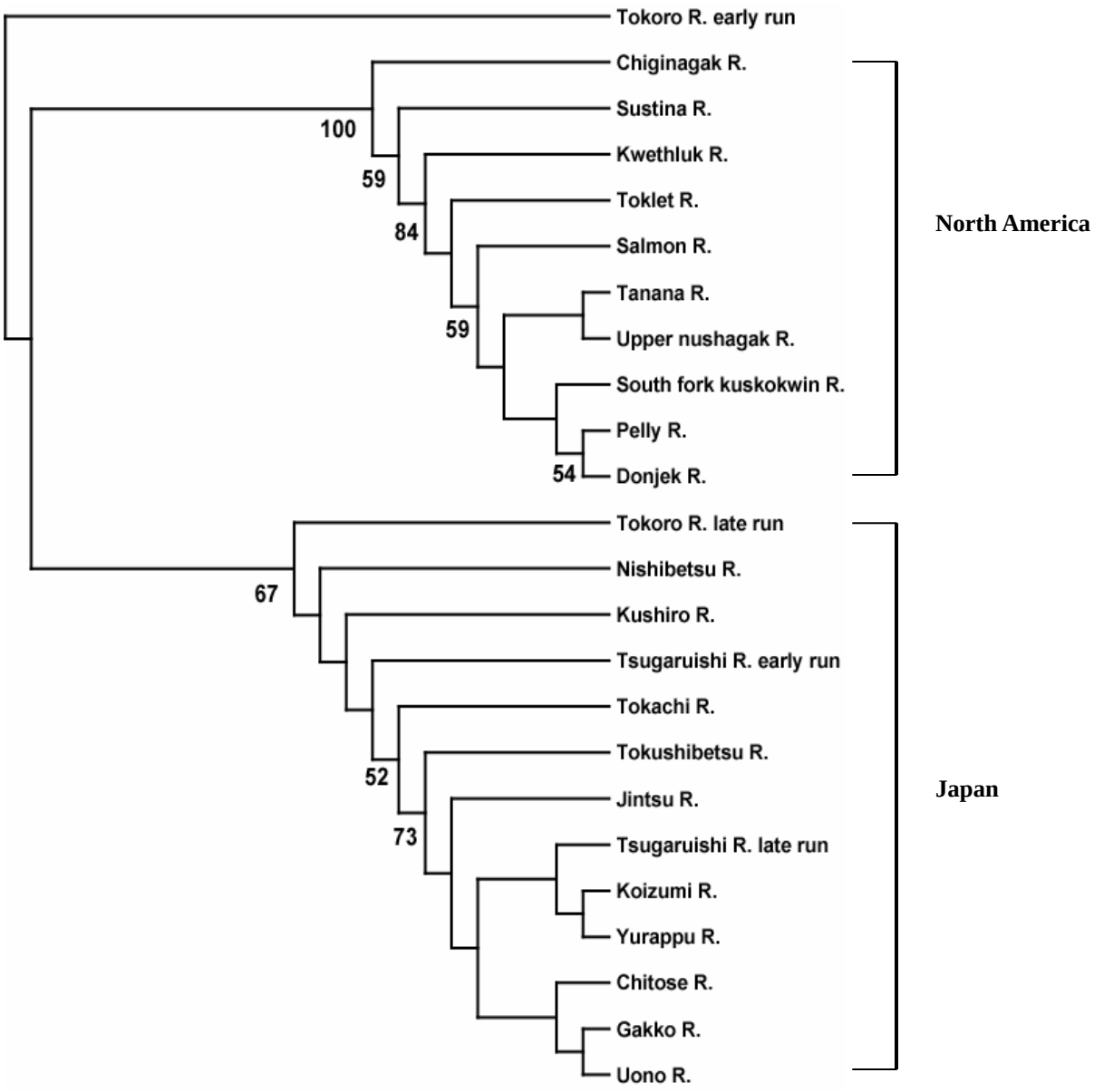

Fig. 3. Neighbor-joining dendrogram inferred from microsatellite data for the 24 chum salmon populations. The topology of the consensus tree (not scaled) is shown with nodal values for bootstrap support over 50% of the 1,000 replicated trees.

Table 1. Additional sampling locations in this study, date of collection, and the numbers of chum salmon samples (N) used for mtDNA analysis

	Sampling location	Date of collection	N
77	Toklet River	1992	50
78	Pelly River	1993	50
79	Donjek River	1994	50
80	South Fork Kuskokwin River	1995	50
81	Olsen River 2004	8 Jun. 2004	50
82	WHN Hatchery	2002	50
83	WHN Hatchery	1992	50
84	Blossom River	1986	50
85	Marten River	1986	50
86	Chunlina, Sustina River	1993	50
87	Joshu Green River	1992	50
88	St. Catherine's Cove	1992	50
89	Frosty Creek	1992	50
90	Little Port Walter	8 Aug 2004	50
91	9 Stream River	8 Jul. 2004	44
92	Vedder River	2002	50
93	Nitinet River	1992	50
94	Harrison River	2002	50
95	Nanaimo River	2002	32
96	Cowichan Bay	1997	50

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	244	250	260	339	340	386	395	401	428	471
A-1*	T	T	A	A	T	T	-	A	C	C	T	C	C	T	A	T	C	G	C	T	A	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	•	•	•	T	•	•	•	•	-	A	•	•	•
B-12*	•	•	•	•	•	•	•	•	G	•	•	•	•	C	•	•	•	-	A	•	•	•
B-13*	•	•	•	•	•	•	•	•	G	•	•	•	•	•	G	•	•	-	A	•	•	•
B-14*	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	A	•	-	A	•	•	•
B-15*	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	-	A	C	•	•
B-16	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	-	A	•	G	•
B-17*	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	•	•	-	A	•		C
B-18*	•	•	•	•	•	•	•	•	G	•	•	•	•	•	•	A	T	-	A	•	•	•
B-19*	•	•	•	•	•	•	•	•	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.
*Cited from Sato et al. (2001, 2004)

Table 3. Distribution of mtDNA control region haplotypes among 20 additional North American populations of chum salmon.

Population	Number of individuals with haplotype																														
no.	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	B18	B19	C1	C2	C3	C4	C5
77	0	0	0	0	0	0	0	0	0	0	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	3	0	10	0	0	0	0	4	0	0	0	0	0
82	0	0	0	0	0	0	0	0	0	0	38	0	2	0	0	0	0	0	3	1	0	6	0	0	0	0	0	0	0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	35	0	4	0	0	0	0	0	6	0	0	5	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0	1	0	0	0	0	14	0	0	0	1	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	34	0	0	0	0	0	0	0	0	0	0	13	0	1	0	2	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0	0	0	41	0	0	0	0	0	0	0	0	0	1	8	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0	46	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
90	0	0	0	0	0	0	0	0	0	0	38	0	0	0	0	0	5	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
91	0	0	0	0	0	0	0	0	0	0	39	0	0	0	0	0	1	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	44	0	2	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	43	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	39	0	3	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	27	0	0	0	0	0	1	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	44	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0

Table 4. Nucleotide divergence among three haplotypes lineages of chum salmon populations.

	Nucleotide divergence	Net Nucleotide divergence	Nucleotide diversity
CladeA and B	0.00740±0.00095	0.00351±0.00096	0.0057
CladeB and C	0.00932±0.00143	0.00533±0.00145	0.0062
CladeA and C	0.00517±0.00119	0.00193±0.00121	0.0042

Table 5. Haplotype diversity (*h*, ±SD) and nucleotide diversity (π, in parentheses) among geographical three regions calculated from mtDNA haplotype frequencies.

Region	Haplotype diversity (*h*)	Nucleotide diversity (π)
Japan	0.607 ± 0.001	0.0021
Russia	0.359 ± 0.001	0.0017
North America	0.174 ± 0.001	0.0005

Table 6. Pairwise F_{ST}.estimates for regional chum salmon populations excluding one Korean population by mtDNA sequence analysis.

	HOK	HON	SEM	KAM	SAK	PRI	MAG	AMU	NWA	SCA	AP	SEA	BCL	WSG
Hokkaido (8)	0.025													
Honshu (8)	0.121	0.038												
Semovoka (1)	0.052	0.034	0.000											
Kamchka (14)	0.550	0.790	0.740	0.023										
Sakhalin (7)	0.515	0.777	0.718	0.018	0.009									
Primory (3)	0.013	0.143	0.056	0.576	0.545	0.002								
Magadan (4)	0.422	0.682	0.621	0.061	0.049	0.440	0.040							
Amur (1)	0.670	0.883	0.842	0.049	0.050	0.731	0.145	0.000						
Northwest Alska(13)	0.698	0.906	0.867	0.092	0.124	0.767	0.194	0.000	0.007					
Southcentral Alaska (10)	0.690	0.899	0.859	0.093	0.109	0.756	0.187	0.017	0.096	0.037				
Alaska Peninsula (5)	0.667	0.883	0.841	0.075	0.095	0.729	0.163	0.009	0.056	0.057	0.033			
Southeast Alaska (8)	0.696	0.905	0.865	0.101	0.117	0.764	0.196	0.014	0.094	0.043	0.049	0.024		
British Columbia (8)	0.689	0.902	0.861	0.103	0.122	0.756	0.192	0.024	0.155	0.042	0.069	0.038	0.025	
Washington (5)	0.695	0.903	0.863	0.106	0.126	0.762	0.198	0.030	0.139	0.047	0.071	0.048	0.038	0.039

Table 7. Results of the hierarchical analyses of molecular variance based on mtDNA for chum salmon. The percentage of variance (%), probability estimated from permutation (*P*), and the F-statistics (Φ) are given at hierarchical level (Excoffier et al. 1992).

Variance component	%	*P*	Φ
Analysis I			
Among three regional groups (Japan, Russia, and North America)	68.7	<0.001	0.68
Among populations within groups	5.0	<0.001	0.16
Within populations	26.25	<0.001	0.74
Analysis II			
Among three regional groups in Japan (Hokkaido, Sea of Japan coast in Honshu, and Pacific Ocean coast in Honshu)	7.8	<0.001	0.08
Among populations within groups	3.2	<0.005	0.03
Within populations	89.0	<0.001	0.11
Analysis III			
Among six regional groups in Russia (Kamchtka, Sakhallin, Primorye, Megadan, Semovodka, and Amur)	29.0	<0.001	0.29
Among populations within groups	1.9	<0.005	0.27
Within populations	69.1	<0.001	0.31
Analysis IV			
Among five regional groups in North America (Northwest Alaska, Alaska Peninsula/Southcentral Alaska, Southeast Alaska, British Columbia, and Washington)	2.1	<0.001	0.02
Among populations within groups	4.1	<0.001	0.04
Within populations	93.8	<0.001	0.06

Table 8. Range of allele size (S), total number of alleles (A_T), the number of samples (N), observed (H_O), expected (H_E) heterozygosity and gene diversity (H_S) by locus for 24 chum salmon populations.

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Chitose River	S	99-119	99-127	67-89	102-160
(N: 54)	A_T	10	12	7	16
	H_O	0.889	0.703	0.407	0.889
	H_E	0.833	0.714	0.572	0.882
	H_S	0.883	0.713	0.562	0.882
Tokushibetsu	S	103-141	101-117	75-105	106-126
River	A_T	11	8	8	12
(N: 31)	H_O	0.903	0.774	0.548	0.839
	H_E	0.819	0.757	0.506	0.872
	H_S	0.817	0.756	0.505	0.872
Tokoro River	S	101-125	99-119	75-85	106-160
(late run)	A_T	12	9	5	14
(N: 43)	H_O	0.651	0.605	0.372	0.791
	H_E	0.708	0.682	0.347	0.861
	H_S	0.709	0.667	0.327	0.853
Tokoro River	S	101-125	101-127	75-83	106-160
(early run)	A_T	10	10	4	13
(N: 41)	H_O	0.610	0.805	0.293	0.781
	H_E	0.705	0.751	0.262	0.889
	H_S	0.691	0.750	0.261	0.887**
Nishibetsu	S	101-121	101-123	75-179	104-132
River	A_T	10	11	10	12
(N: 58)	H_O	0.586	0.741	0.603	0.672
	H_E	0.671	0.798	0.545	0.870
	H_S	0.671	0.792	0.544	0.868**
Kushiro River	S	105-125	101-125	75-85	106-162
(N: 45)	A_T	10	10	5	13
	H_O	0.733	0.578	0.244	0.822
	H_E	0.750	0.672	0.264	0.864
	H_S	0.739	0.673	0.264	0.861
Tokachi River	S	101-121	101-123	75-101	106-164
(N: 57)	A_T	10	12	8	15
	H_O	0.737	0.719	0.298	0.825
	H_E	0.804	0.729	0.301	0.885
	H_S	0.804**	0.720	0.301	0.885
Yurappu River	S	105-119	99-123	71-89	106-160
(N: 48)	A_T	8	11	7	11
	H_O	0.708	0.708	0.438	0.771
	H_E	0.785	0.740	0.553	0.844
	H_S	0.786	0.731	0.540	0.845
Tsugaruishi	S	105-117	101-123	75-91	106-160
River (late run)	A_T	7	10	7	10
(N: 58)	H_O	0.862	0.759	0.483	0.759
	H_E	0.784	0.751	0.447	0.833
	H_S	0.779	0.751	0.447	0.833

Table 8. (continued)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
Tsugaruishi	S	103-129	99-123	75-91	106-148
River (early run)	A_T	10	12	7	3
(N: 44)	H_O	0.727	0.659	0.386	0.886
	H_E	0.789	0.758	0.387	0.866
	H_S	0.790	0.749	0.387	0.865
Koizumi River	S	107-125	99-119	75-91	106-124
(N: 19)	A_T	6	9	5	8
	H_O	0.895	0.737	0.632	0.684
	H_E	0.802	0.784	0.542	0.804
	H_S	0.795	0.785	0.539	0.782
Gakko River	S	101-119	101-127	75-91	106-160
(N: 47)	A_T	10	12	7	12
	H_O	0.830	0.681	0.617	0.702
	H_E	0.830	0.635	0.618	0.868
	H_S	0.830	0.622	0.606	0.865**
Uono River	S	101-123	101-123	73-83	104-160
(N: 49)	A_T	12	11	5	13
	H_O	0.816	0.551	0.592	0.776
	H_E	0.857	0.678	0.488	0.839
	H_S	0.852	0.655**	0.487	0.839
Jintsu River	S	103-129	99-127	75-87	106-160
(N: 49)	A_T	9	12	8	15
	H_O	0.735	0.796	0.510	0.816
	H_E	0.799	0.777	0.557	0.876
	H_S	0.800	0.777	0.558	0.869
Kwethluk River	S	99-125	101-119	65-83	100-120
(N: 46)	A_T	10	9	3	8
	H_O	0.457	0.457	0.000	0.435
	H_E	0.535	0.588	0.185	0.611
	H_S	0.533	0.588	0.165**	0.592**
Toklet River	S	109-121	101-127	75-79	106-112
(N: 46)	A_T	4	10	2	2
	H_O	0.413	0.739	0.065	0.391
	H_E	0.486	0.688	0.085	0.451
	H_S	0.482	0.688	0.064	0.445
Tanana River	S	109-127	101-115	79-85	106-114
(N: 49)	A_T	8	6	2	5
	H_O	0.347	0.286	0.000	0.592
	H_E	0.555	0.344	0.099	0.590
	H_S	0.511	0.294	0.041	0.563
Upper	S	103-121	101-117	79	106-112
Nushagak River	A_T	6	6	1	4
(N: 49)	H_O	0.531	0.265	monomorphic	0.469
	H_E	0.530	0.296		0.541
	H_S	0.530	0.278	0.000	0.542

Table 8. (continued)

Population		Locus			
		OKM4	OKM5	OKM7	OKM8
South Fork	S	107-121	101-117	75-79	100-120
Kuskokwin	A_T	5	6	2	6
River	H_O	0.596	0.213	0.021	0.511
(N: 47)	H_E	0.623	0.291	0.042	0.638
	H_S	0.611	0.273**	0.021	0.606**
Salmon River	S	99-125	101-117	75-79	106-126
(N: 48)	A_T	8	7	2	5
	H_O	0.438	0.354	0.021	0.417
	H_E	0.499	0.351	0.120	0.454
	H_S	0.484	0.334	0.101	0.440
Donjek River	S	107-121	101-113	79	106-112
(N: 42)	A_T	5	3	1	3
	H_O	0.500	0.333	monomorphic	0.571
	H_E	0.581	0.372		0.532
	H_S	0.545	0.316	0.000	0.479
Pelly River	S	109-123	101-119	75-79	106-112
(N: 45)	A_T	5	4	2	2
	H_O	0.711	0.089	0.022	0.600
	H_E	0.586	0.266	0.127	0.559
	H_S	0.585	0.170**	0.022	0.488
Chunlina,	S	105-125	101-119	69-79	106-112
Sustina River	A_T	7	7	2	3
(N: 46)	H_O	0.739	0.630	0.022	0.630
	H_E	0.762	0.712	0.043	0.541
	H_S	0.757	0.704	0.022	0.540
Chiginagak	S	105-125	95-119	71-83	102-132
River	A_T	9	10	6	11
(N: 44)	H_O	0.386	0.500	0.068	0.705
	H_E	0.680	0.759	0.364	0.725
	H_S	0.682**	0.760**	0.346**	0.714**
	S	99-141	95-127	65-179	100-164
Total (all sites)	A_T	17	16	17	29
	H_O	0.658	0.570	0.277	0.681
	H_E	0.699	0.621	0.310	0.737
	H_S	0.692	0.606	0.296	0.726

*Departure from Hardy-Weinberg equilibrium by Markov chain procedure (P<0.01)

Table 9. Results of the hierarchical analyses of molecular variance based on msDNA for chum salmon. The percentage of variance (%), probability estimated from permutation (P), and the F-statistics (Φ) are given at hierarchical level (Excoffier et al. 1992).

Variance component	%	P	Φ
Analysis I			
Among regional groups (Japan and North America)	9.93	<0.001	0.10
Among populations within groups	2.73	<0.001	0.03
Within populations	87.34	<0.001	0.13
Analysis II			
Among regional groups in Japan (Hokkaido, Sea of Japan coast in Honshu, and Pacific Ocean coast in Honshu)	0.44	>0.05	0.004
Among populations within groups	1.52	<0.001	0.02
Within populations	98.04	<0.001	0.02
Analysis III			
Among regional groups in North America (Northwest Alaska, Southcentral Alaska, and Alaska Peninsula)	8.27	<0.001	0.08
Among populations within groups	2.54	<0.001	0.03
Within populations	89.19	<0.001	0.11