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Thesis for the Degree of Master of Science

Genetic stock identification of chum salmon (*Oncorhynchus keta*) collected from the western Bering Sea during summer-autumn 2004

By

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Department of Marine Biology

The Graduate School

Pukyong National University

February 2007

Genetic stock identification of chum salmon (*Oncorhynchus keta*) collected from the western Bering Sea during summer-autumn 2004

2004년 베링해에서 채집된 연어 (*Oncorhynchus keta*)의 유전적 조성에 관한 연구

Advisor: Prof. Suam Kim

by
Min Ho Kang

A thesis submitted in partial fulfillment of the requirements
for the degree of

Master of Science

in Department of Marine Biology, The Graduate School,
Pukyong National University

February 2007

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ABSTRACT

Population structure of chum salmon (*Oncorhynchus keta*) was examined using mtDNA of 3,266 specimens collected from 76 populations along the Pacific rim. Both phylogenetic tree and AMOVA test indicated that the entire populations can be re-grouped as four regional groups (Korea-Japan-Primorie, Russia, Northwest Alaska, and other North America). Also, in order to determine the regional proportions of ocean mixtures, I examined mtDNA of chum salmon collected from the western Bering Sea during Sept. 26 ~ Oct. 23, 2004. The Statistics Program for Analyzing Mixtures (SPAM) estimation was

performed for regional stock contribution using maximum likelihood algorithm. In fall 2004, stock composition in the wetern Bering Sea was 42% Korea-Japan-Primorie, 41% Russian, 13% Northwest Alaskan, and 4% other North American groups.

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INTRODUCTION

1. Distribution of chum salmon in the North Pacific

There are seven species of Pacific salmon, Chinook (*Oncorhynchus tshawytscha*), chum (*O. keta*), coho (*O. kisutch*), pink (*O. gorbuscha*), sockeye (*O. nerka*), cherry (*O. masou*), and steelhead (*O. mykiss*) in the Pacific Ocean (Groot and Margolis, 1991). Six of them, excluding cherry salmon, migrate to the feeding grounds in the ocean, and they begin to grow to the final size during their ocean life period. Anadromous chum salmon, commercially valuable salmonid species in the North Pacific nations, are known for their extensive oceanic migrations. Chum salmon are well known to reside for 3~5 years until they are mature and then, they return to their natal river to spawn. Many studies have been already investigated for early life history (Salo, 1991) and feeding behavior of chum salmon (Hansen and Quinn, 1998). In addition, documents on the distribution and migration patterns of chum salmon have been reported continually based on a long-term accumulation of high-seas tagging or specific genetic data.

Recently, Urawa (2000) and Urawa *et al*. (2001) suggested a migration model of Japanese chum salmon. Seo et al. (2006) modified Urawa's model for

migration of Korean chum salmon. For the eastern Pacific salmon, Beamish et al. (2005) reviewed migratory patterns of pelagic fishes including Pacific salmon related to the aspect of energy between open ocean and coastal ecosystem. The high-seas tagging studies conducted by the National Marine Fisheries Service (NMFS) of the USA. suggested an outline of migration routes of chum salmon in the North Pacific. Those studies showed that there was overlap of ocean ranges of Asian and North American chum salmon in the Bering Sea and around the Aleutian Islands (Seeb *et al.,* 2004, Fig. 1). In addition, Gritsenko (2002 and 2003) examined morphologic, genetic, parasitologic, and tagging methods for stock identification of salmon stocks in the sea, and reported that Asian and American chum salmon were distributed in the US EEZ in the Aleutian waters, and in the Bering sea east of donut and in some extent, in the Gulf of Alaska with a high degree of overlap.

In 2002-2005, scientific surveys were carried out in the Bering Sea to understand the mechanisms of how changes in ocean conditions affect the survival and growth of salmon. Surveys were conducted and coordinated using Russian R/V Tinro by scientists of the member nations of the North Pacific Anadromous Fish Commission (NPAFC) under the Bering-Aleutian Salmon International Survey (BASIS) program (NPAFC, 2004).

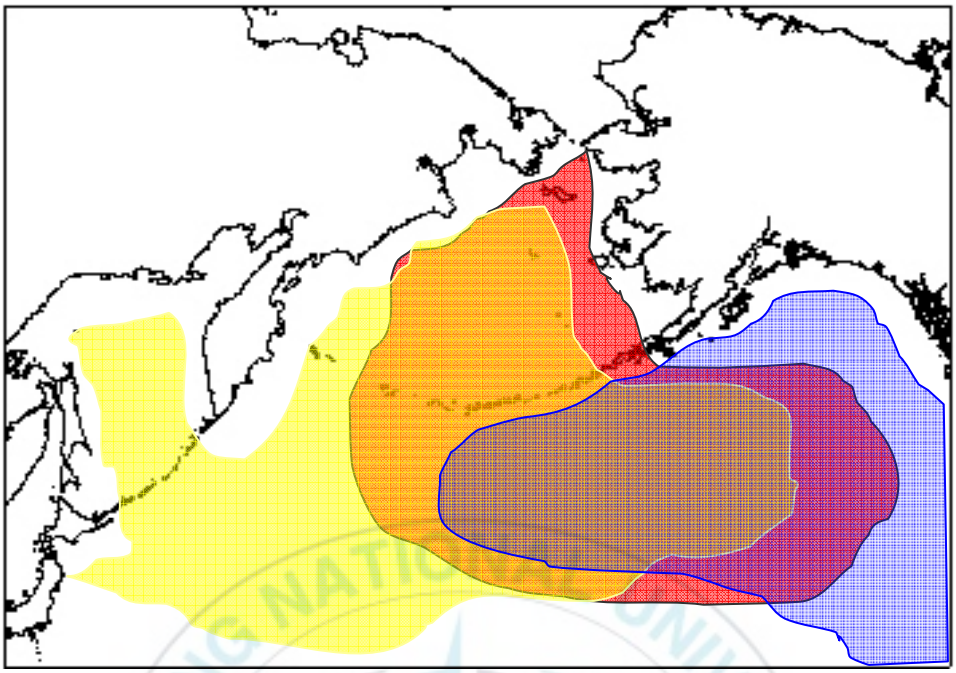

Fig. 1. Schematic boundaries of chum salmon distribution originated from Asia (Korea, Japan, and Russia; Yellow color), western Alaska (Red color), and other North America (Blue color) (modified from Urawa, 2000; Beamish et al., 2005; Seeb *et al.,* 2004).

2. DNA marker technologies in stock identification for chum salmon

Besides issues of survival and growth of salmon, questions about distribution, intermixing, ocean migration, and identification of stock have been studied by salmon scientists under the BASIS program. Recently, the detatils of their distribution patterns and migrations at sea have been studied by using various attempts inferred from catch data, tagging experiments, parasite studies, etc. Specifically, chemical analysis of otolith has been used for stock identification of Pacific chum salmon (Kang, 2003).

Stock identification of chum salmon has been also carried out based on DNA-based analysis such as Microsatellite DNA (Beacham *et al.,* 2003), single nucleotide polymorphism (SNPs, Seeb *et al.,* 2005), mitochondrial DNA (Sato *et al.,* 2004; Yoon *et al.,* 2005; Lee *et al.,* 2005). Genetic stock identification (GSI) was determined in mixed fisheries species as well as genetic population structure of Pacific salmon. As noted by Latham *et al.* (2004), scale-based stock identification for sockeye salmon was improved when each sockeye's scale data were matched to its DNA-based stock, indicating that DNA analysis accompanied with other stock identification methods will allow more accurate and precise estimates in stock identification.

3. Objectives

For genetic stock identification studies of chum salmon, various baseline data such as allozyme (Urawa and Ueno, 1997 and 1999; Urawa *et al.,* 1997 and 1998; Kondzela *et al.,* 2002; Seeb *et al.,* 2004), mitochondrial DNA (Sato *et al.,* 2004; Yoon *et al.,* 2004), microsatellite DNA (Beacham, 1996; Beacham *et al.,* 2003) have been analyzed. Especially, Sato *et al*. (2004) and Yoon *et al*. (2004) chose SNPs in about 500bp of the variable portion of the mtDNA control region to build a baseline data for chum salmon, and they examined the genetic variation and their population structure of about 3,300 specimens collected from 76 populations along the Pacific Rim.

The aim of this study is to investigate genetic stock identification of chum salmon mixtures in the western Bering Sea based on the previously analyzed SNPs baseline data (Yoon *et al.,* 2004). Thus, this study will increase our understanding on salmon distribution and migration patterns in the North Pacific ocean.

MATERIALS AND METHODS

1. Re-analyzed Baseline data

1.1 Sampling locations for baseline data

Baseline data cited from Yoon *et al.* (2004) have provided opportunities to analyze haplotype and nucleotide diversity of Pacific chum salmon, and these data were also used for identification of mixed salmon stock in the high-seas. The geographical locations of chum salmon collected from Korea (1 population), Japan (16 populations), Russia (30 populations), Northwest Alaska (9 populations), and other North America (20 populations) are presented in Fig. 2 and Appendix Ⅰ.

1.2 Analytical method for baseline data

For baseline data analysis, the nucleotide sequences of 3,266 previously examined individuals were re-analyzed in this study (Appendix Ⅰ and Fig. 2). The sequences were re-aligned and compared using the DNASIS version 2.5 program (Hitachi Software Engineering Co., Ltd.). Phylogenetic tree was constructed for each replicate estimated by the neighbor-joining method (Saitou and Nei, 1987) using the NEIGHBOR program in PHYLIP (Felenstein, 1993).

Genetic differentiation among populations at different levels of geographic hierarchy was quantified by analysis of molecular variance (AMOVA, Excoffier *et al.,* 1992). Variance components and F*st* values were also estimated within and among four hierarchical levels (Korea-Japan-Primorie, Russia, Northwest Alaska, and other North America)

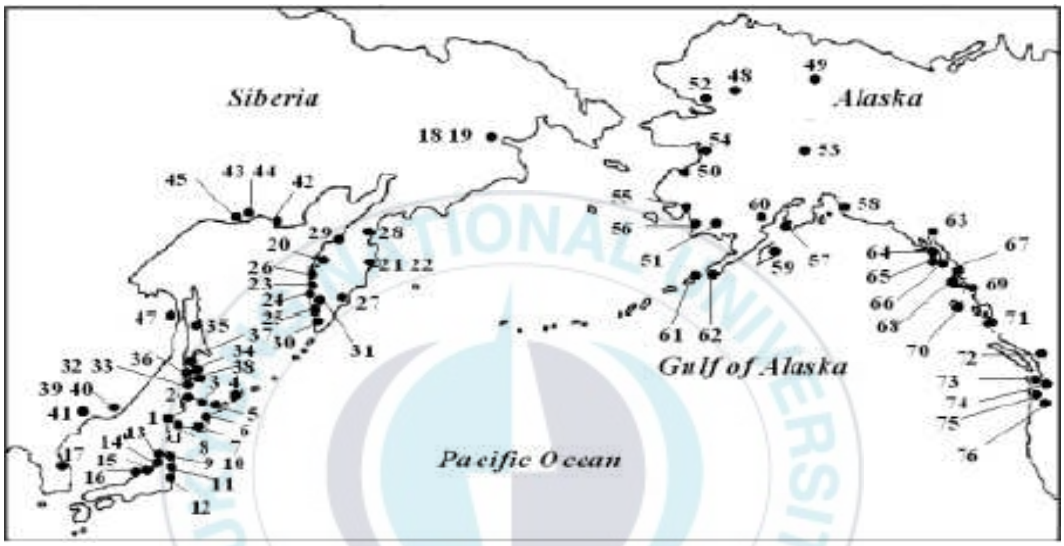

Fig. 2. Sampling area of 76 chum salmon populations for baseline data in the Pacific Rim (Yoon *et al.,* 2004).

2. Mixed-stock data

2.1 Fish samples

Immature chum salmon were collected from the western Bering Sea during summer-autumn period of 2004 caught by the R/V TINRO research cruise (Fig. 3). One-hour trawl operation was made in the intermediate layer using a mid-water trawl. The speed of trawl towing varied within the range of 3.9 - 5.1 knots (on the average 4.4 knots). A total of 826 chum salmon were sampled at 48 stations, and parts of fin-ray from individual salmon preserved in 5% alcohol. However, data from 23 stations that had more than 15 specimens per trawl were only used for this study (Table 1). Tissue samples were kept -80℃ until DNA extraction.

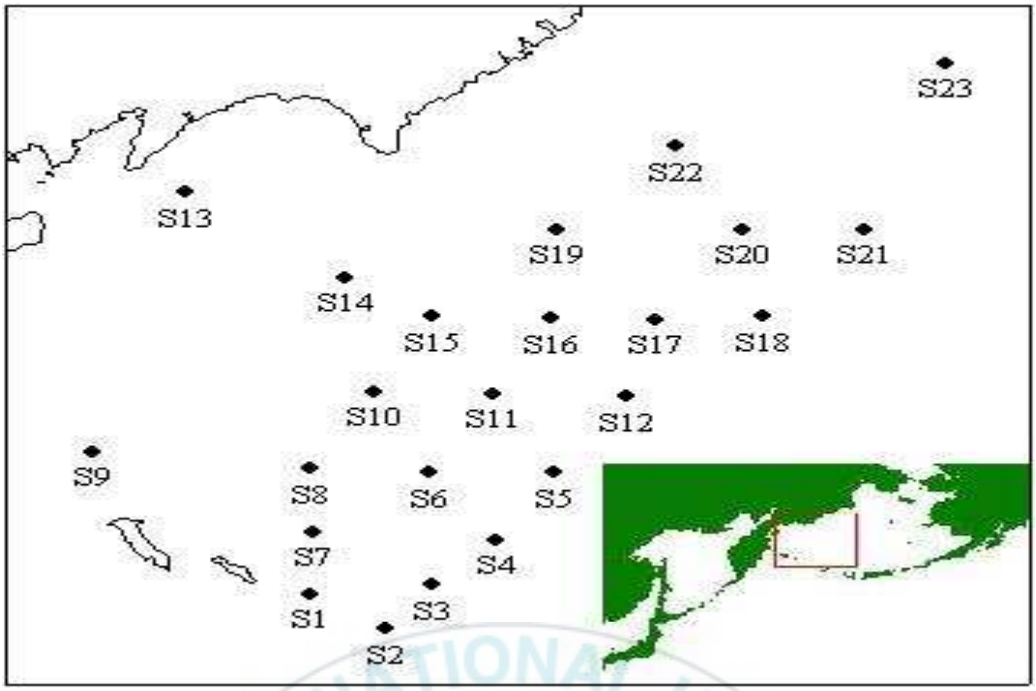

Fig. 3. Sampling locations of mixtures in the western Bering Sea during Sept. 26 - Oct. 23, 2004.

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Table 1. Sampling locations with the latitudes and the longitudes, date of collections, and the number of samples.

Station No.	Location		Date of collection	N
	Latitude	Longitude		
S1	54°20'12"N	169°02'30"W	Sept. 26	23
S2	53°52'24"N	170°16'30"W	Sept. 26	29
S3	54°29'12"N	171°01'60"W	Sept. 27	30
S4	55°04'48"N	172°03'42"W	Sept. 27	16
S5	55°58'42"N	172°58'30"W	Sept. 27	18
S6	55°58'42"N	170°58'54"W	Sept. 28	30
S7	55°11'21"N	169°05'30"W	Sept. 28~29	42
S8	56°02'18"N	169°01'60"W	Sept. 30	30
S9	56°15'00"N	165°30'00"W	Oct. 1~3	23
S10	57°00'54"N	170°04'00"W	Oct. 4	28
S11	56°59'24"N	172°01'24"W	Oct. 4	25
S12	56°58'30"N	174°09'48"W	Oct. 5	28
S13	59°30'00"N	167°00'12"W	Oct. 7~10	32
S14	58°27'45"N	169°36'27"W	Oct. 11	34
S15	57°58'30"N	171°01'60"W	Oct. 12	46
S16	57°57'36"N	172°57'06"W	Oct. 12	49
S17	57°55'42"N	174°38'54"W	Oct. 13	34
S18	57°55'42"N	176°22'06"W	Oct. 13	48
S19	59°02'24"N	173°03'39"W	Oct. 14~16	33
S20	59°01'54"N	176°02'24"W	Oct. 16	45
S21	59°02'36"N	178°03'06"W	Oct. 17	52
S22	60°02'06"N	174°57'21"W	Oct. 17~19	20
S23	60°59'30"N	179°21'12"W	Oct. 19~23	23

2.2 DNA extraction and PCR amplification

The mtDNA was isolated from the tissue samples (fin) using a modification of the Non-idet method described by Champman and Powers (1984). The tissue were routinely homogenized in its 5 volumes of 0.25 M sucrose-TEK (TEK; 50 mM Tris, 10 mM EDTA, 1.5% KCL, pH 7.5) buffer using a single storke of a motor-driven glass Teflon homogenizer. Nucleic and cell debris were removed by centrifugation at 1,000×g for 10 min at 4℃. The supernatant is drawn off and mitochondria pelleted by centrifugation at 18,000×g for 1 hr at 4℃. The mitochondrial pellet is resuspended on 0.9 ml TEK per 2.5 g starting tissue and placed in a sterile microcentrifuge tube. The suspension is then made to 1% Non-idet by addition of a 10% Non-idet-TEK solution to lyse the mitochondrial membranes. The suspension is then centrifuged at 12,000×g for 10 min to remove debris. The remainder of the produce followed Sambrook *et al.* (1989) as detailed below. The supernatant containg mtDNA was extacted with an equal volume of phenol : chloroform : isoamyalcohol (25 : 24 : 1), the mtDNA then was precipitated by adding 0.1 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of cold ethanol. The mtDNA from 1 g starting tissue was dissolved in 100 ㎕ of sterile water and stored at -20℃.

Mitochondrial control region was amplified by the polymerase chain reaction (PCR) using a standard protocols (McVeigh *et al.,* 1991). PCR amplification was performed with 0.2 - 0.5 ㎍ of template DNA in a reaction mixture of 50 ㎕ containing 1.25 units of Taq DNA polymerase (Ex TaqTM, TAKARA), 0.2 mM of each dNTP and 0.5 mM of each primer. Thirty-five PCR cycles were performed : preheating at 95℃ for 5 min, followed by 35 cycles of denaturation at 94℃ for 1 min, annealing at 54℃ for 1 min, and elongation at 72℃ for 1 min, and completed with the elongation at 72℃ for 5 min. Oligonucleotide primer pairs were used PRO-L (5'-CTACCTCCAACTCCCAAAGC-3') and DL-2H (5'-TAGGGTCC(A/G)TCTTAACAGCTTCA-3').

2.3 Direct sequencing of PCR products

PCR products were purified with QIAquick spin columns. The products were subjected to generate templates for cycle sequencing. The nucleotide sequences were determined with the dideoxy chain-termination method using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Corp., Norwalk, USA). In cycle sequencing, 25 cycles of 96℃ for 10 sec; 50℃ for 5 sec; 60℃ for 4 min were used. Excess terminators were removed by ethanol precipitation. The samples were analyzed with an automated DNA sequencer

(ABI PRISM 377). Further details were according to the manufacturer's recommendations.

2.4 Analytical method for mixed-stock data

Haplotype frequencies were determined at each locus in a total of 76 populations and Statistic Program for Analyzing Mixtures (SPAM version 3.7, ADFG 2003) (Debevec *et al.,* 2000) was used to estimate stock composition of mixtures in the western Bering Sea specimens. Estimates of stock contributions were made with a conditional maximum likelihood algorithm (Pella and Milner, 1987) and precision of the composition estimates was calculated by bootstrap resampling (Efron and Tibshirani, 1986). Estimated stock composition of a simulated mixture was determined, and the whole process was repeated 100 times to estimate the mean and standard deviation of the individual stock composition estimates. Estimates were made to individual stocks and then pooled to regional stock groups: Japan, Russia, Northwest Alaska, and other North America. These regional stock groups were categorized based on previous genetic analysis for the baseline data set of 76 populations of chum salmon in the Pacific Rim (Yoon *et al.,* 2004; Sato *et al.,* 2004).

RESULTS

1. Distribution of mtDNA haplotypes in chum salmon along the Pacific-rim

The results from sequence analysis of chum salmon have revealed that nucleotide sequence analysis of about 500bp in the hypervariable regions of the 5' end of the mtDNA control region identified 20 variable nucleotide sites from the 30 haplotypes and classified three geneological groups of clade A, B, and C among 3,266 individuals of 76 Pacific-rim populations (Yoon *et al.,* 2004). Distribution of 30 haplotypes among 76 populations of chum salmon is tabulated in Appendix Ⅱ.

Sato *et al.* (2004) and Yoon *et al.* (2004) showed that some of the haplotypes categorized Pacific-rim populations of chum salmon into regional groups in Fig. 4. Clade B and C haplotypes occurred mostly in Russian populations, except for Taranay and Okhotske population, where the clade B haplotypes only appeared. In addition, A-1 haplotype appeared in Primorie region (Narva, Avakumovka and Avakumovka early run population) and Belaya population from Sakhalin. Clade B haplotypes were the most common sequences in North American populations. However, relatively different distribution of clade B haplotypes was observed between Northwest Alaskan populations and other North American populations

(Peninsula/Southcentral/Southeast Alaska, Canada, and Washinton). Haplotype B-3 was the most common sequence between them. On the other hand, haplotype B-13 only occured in other North American populations, except for two populations in Southcentral Alaska (Kitoi Hatchery and McNeil River). In Korean and Japanese populations, relatively rich clade A and C haplotypes occured.

The observed haplotypes were mostly associated with geographic regions in that clade A and C haplotypes were represented by Korean and Japanese populations, clade B and C haplotypes by Russian populations, haplotype B-3 by Northwest Alaskan populations, and haplotype B-3 and B-13 by other North American populations (Fig. 4).

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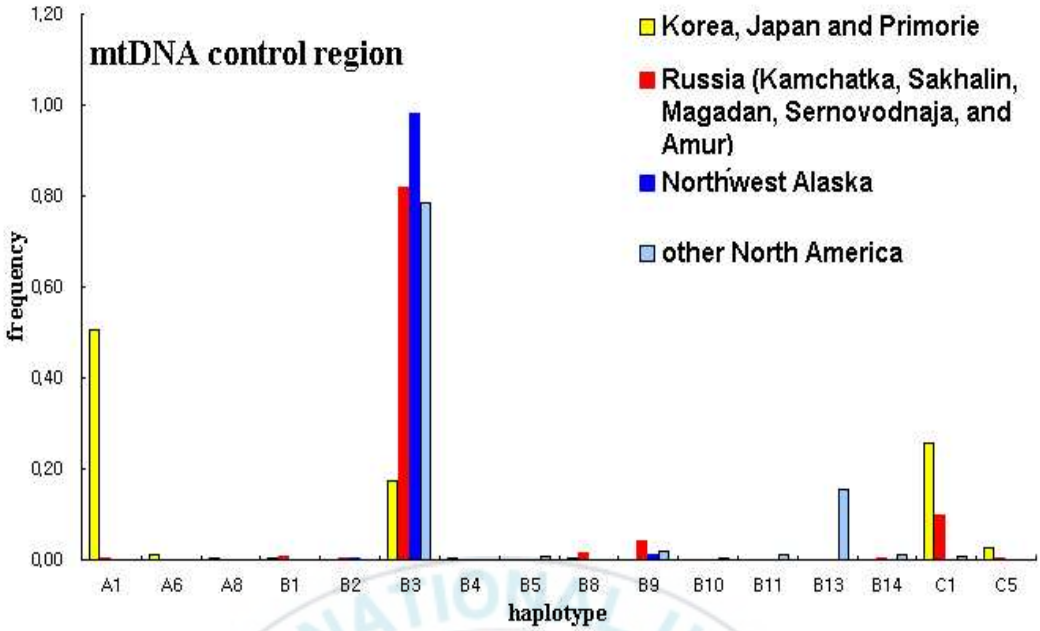

Fig. 4. Frequency distribution of chum salmon mtDNA haplotypes given for four major geographic locations.

2. Geographic differentiation in the Pacific-rim populations

Using the genetic distance estimates of Kimura-two parameters, a possible Neighbor-joining tree of 76 populations separated the Korean-Japanese-Primorie, Russian, Northwest Alaska, and other North American groups in Fig. 5. The North American populations were devided by Northwest Alaskan populations and other North American populations. There was a separation within Russian populations. Three subpopulations in Primorie region (Narva, Avakumovka, and Avakumovka early run population) were located within Korean and Japanese populations.

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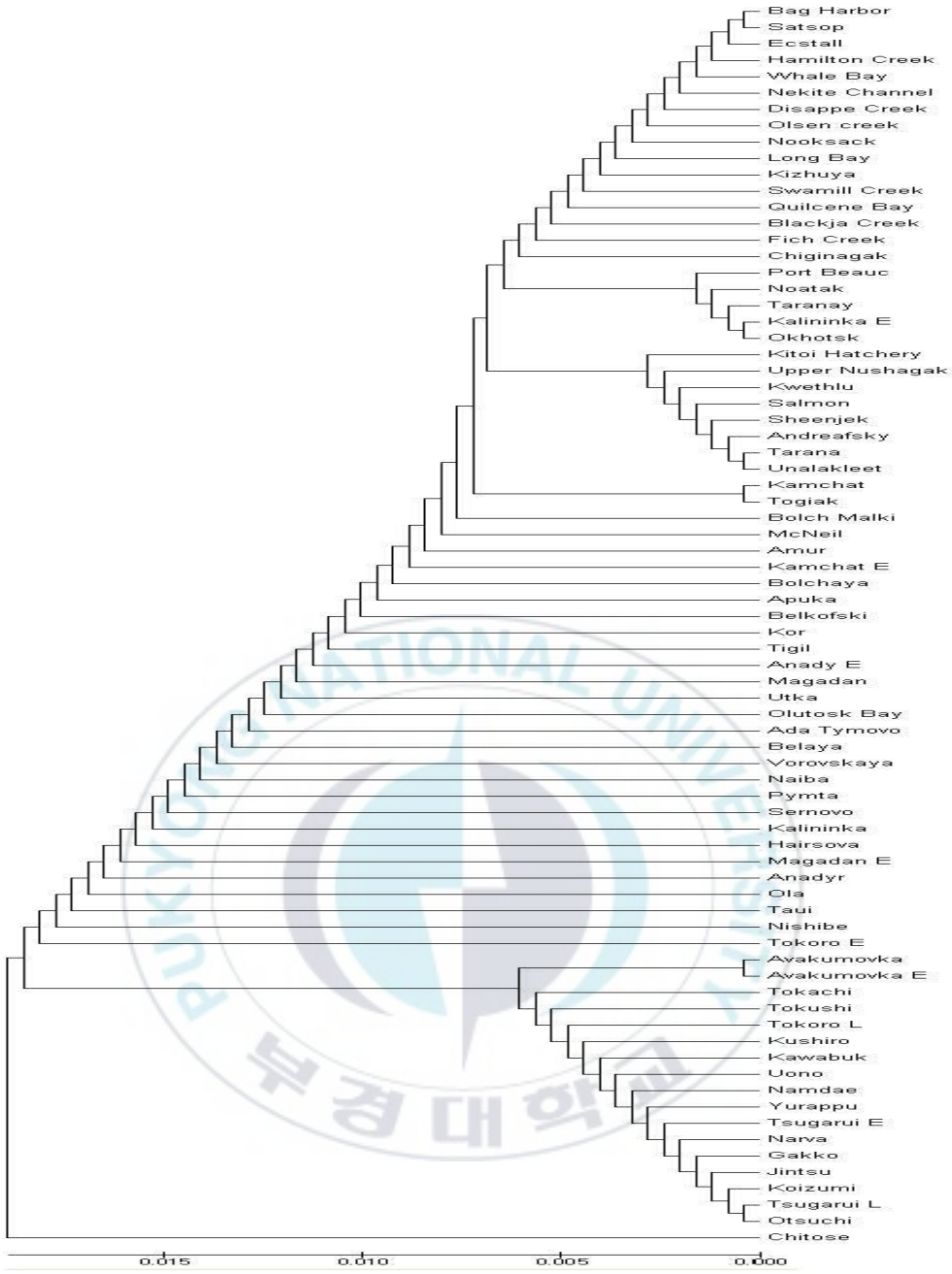

Fig. 5. Neighbor-joining distance tree for the mtDNA control region of chum salmon from the Pacific-rim populations.

In order to evaluate the reliability of the inferred Neighbor-joining tree, analysis of molecular variance (AMOVA, Excoffier *et al.,* 1992) was simulated in the Pacific-rim populations. As noted by Yoon (2004), AMOVAs (Table 2) revealed the following populations structure in chum salmon; very strong geographic structuring among Japan, Russia, and North America (56.8% of the total variance, $p<0.001$, Analysis Ⅰ), as compared with the average extent of structuring among populations within each geographic group (6.2% of the total variance); similar level of population structuring among three regional groups in Japan (7.6% of the variance, $p<0.001$, Analysis Ⅱ), among five regional groups in North America (7.0% of the variance, $p<0.001$, Analysis Ⅳ), and among six regional groups of Russia (25.35% of the variance, $p<0.001$, Analysis Ⅲ). However, re-analyzed AMOVA also indicated that the higher degree of variation occurred and there was a highly significance ($p<0.001$) in the probability test among four regional groups (Analysis Ⅴ, Table 2).

Table 2. Results of the hierarchical analyses of molecular variance for chum salmon. Analysis Ⅰ- Ⅳ were cited from Yoon *et al.* (2004), and Analysis Ⅴ from this research.

Variance component	Percentage of variance (%)	Probability (P)	F-statistics (Φ)
Analysis Ⅰ			
Among three regional groups (Japan, Russia, and North America)	56.83	<0.001	0.68
Among populations within groups	6.2	<0.001	0.0075
Within populations	37.0	<0.001	0.44
Analysis Ⅱ			
Among three regional groups in Japan (Hokkaido, Japan/East sea coast, Pacific ocean coast)	7.6	<0.001	0.064
Among populations within groups	1.8	<0.05	0.015
Within populations	90.7	<0.001	0.77
Analysis Ⅲ			
Among six regional groups in Russia (Kamchtka, Sakhallin, Primorye, Magadan, Sernovodnaja, and Amur)	25.35	<0.001	0.20
Among populations within groups	2.16	<0.001	0.02
Within populations	72.49	<0.001	0.60
Analysis Ⅳ			
Among five regional groups in North America (Northwest Alaska, Alaska Peninsula/Southcentral Alaska, Southeast Alaska, British Columbia, Washington)	7.06	<0.001	0.012
Among populations within groups	3.85	<0.001	0.006
Within populations	89.09	<0.001	0.145
Analysis Ⅴ			
Among four regional groups (Korea-Japan-Primorie / Russia / Northwest Alaska / other North America)	35.95	<0.001	0.410
Among populations within groups	5.04	<0.001	0.079
Within populations	59.01	<0.001	0.360

3. Haplotype composition and distribution of chum salmon in the western Bering Sea

In order to evaluate the genetic stock identification of chum salmon mixtures, I analyzed mtDNA haplotypes of 738 specimens at 23 stations in the western Bering Sea (53°53' to 60°59' N, 167°00' to 179°21' W, Table 1). As a result, a total of 18 haplotypes were detected with 13 segregating sites in the 481bp 5' hypervariable portion of the mtDNA control region (Table 3).

A-1, B-3, and C-1 haplotypes mainly appeared in all the stations and they were focal haplotypes in three clades. Haplotype frequency distribution were different among the stations. The clade B and C haplotypes, which represents Russian stocks, were observed with the higher compositions up to ca. 87% in the stations, except for S6 and S12. The clade A and C haplotypes, which represents Korean and Japanese stocks, were predominant in five stations (S15, S17, S21, S22, and S23). Particularly, two stations (S17 and S23) constitute ca. 65% with the highest peaks of the graph (Fig. 6). Whereas the clade B haplotypes were clearly distinctive with the higher compositions of haplotype B-3 (Table 3). The relative frequency of haplotype B-3 mostly showed the tendency to have the highest values in the stations, except for S23.

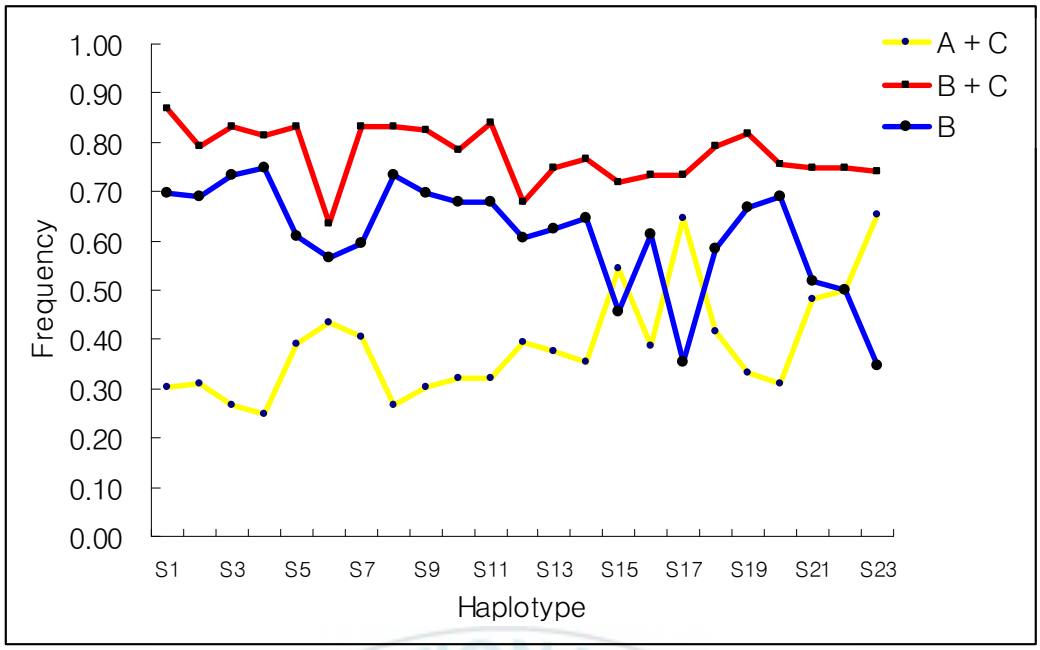

Fig. 6. Relative frequency of chum samon haplotype distribution among 23 stations in the western Bering Sea.

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Table 3. Haplotype distribution of chum salmon among 23 stations in the western Bering Sea.

Station	A1	A3	A4	A5	A6	A8	B1	B2	B3	B6	B8	B9	B11	B12	B15	C1	C3	C5
S1	3						1		14			1				4		
S2	6						2		17			1				2		1
S3	5						1		21							3		
S4	3								11	1						1		
S5	2		1				1		10							4		
`S6	11						1		15		1					2		
S7	7						1		21			2			1	9		1
S8	5						1		21							3		
S9	4						1		14		1					3		
S10	6								19							3		
S11	3			1					17							4		
S12	9						3		13		1					2		
S13	8						1		17			2				4		
S14	6				1	1	4		18							4		
S15	13								18		1	2				12		
S16	13						2		25		2			1		5		1
S17	9								12							12	1	
S18	9				1		1		27							10		
S19	6						2		19				1			5		
S20	11						1	1	28					1		3		
S21	11	2					1	3	22			1				12		
S22	5								10							5		
S23	4				2			2	6							9		

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4. Stock composition of mixtures in the western Bering Sea

The maximum likelihood analysis of mixtures provided accurate estimates of regional proportions comprised of simulation studies of four regions, Korea-Japan-Primorie (JKP), Russia (RU), Northwest Alaska (NA), and other North America (OAN). In simulation studies where the true regional contributions were above 90%, the average of maximum likelihood estimates was more than 97.4% accurate.

The regional proportions of mixtures from S15 to S23 in the northern parts of the western Bering Sea were estimated at 35.2-69.9% JKP, 26.8-48.7% RU, 1.8-18.9% NA, and 0.1-17.8% OAN (Fig. 7; Table 4). Thus, while regional proportions were dominated by JKP and RU stocks, the estimates of OAN indicated a low level of contributions (< 4%) except for S19 (17.8%). The stock components of three stations (S9, S13, and S14) in the vicinity of the Russian coast were dominated by 52.9-54.9% RU and 32.9-40.8% JKP stocks, while the stock components of NA were ranged from 3.1 to 10.1% with smaller contribution by OAN stock (1.2-3.3%). In the rest parts of the western Bering Sea (S1 - S8, S10 - S12), JKP stock made up 25.6-49.7% and RU stock constituted 24.8-56.0%, with smaller contributions of OAN (0.6-8.6%). In contrast, the stock components of NA were higher than those of other stations in the western Bering

Sea, except for S12.

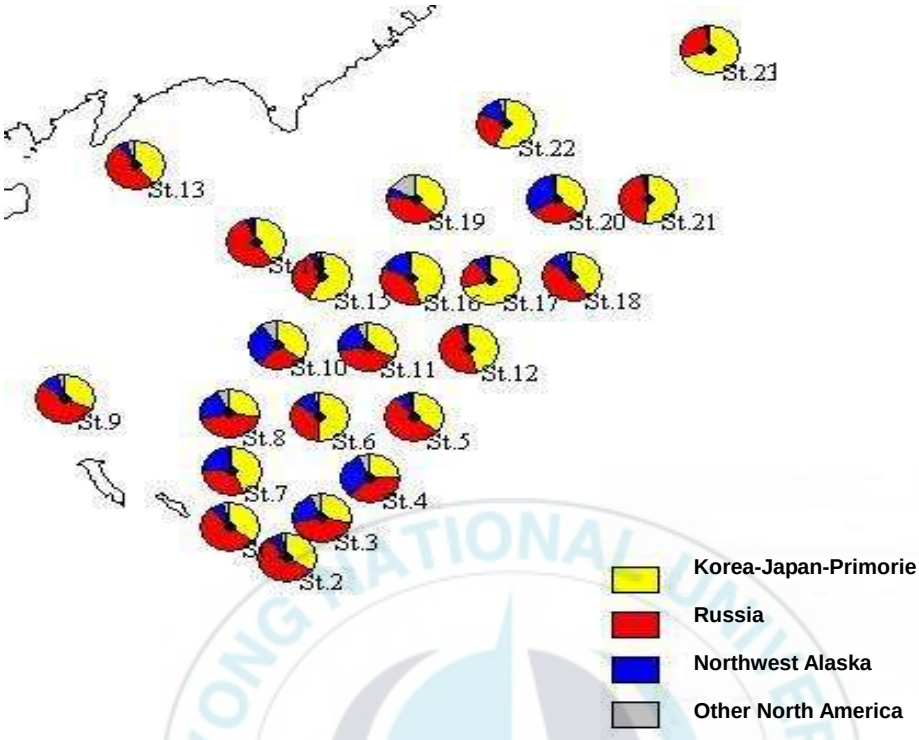

Fig. 7. Genetic Stock Identification (GSI) using the estimated regional proportions of mixtures in the western Bering Sea.

Table 4. Estimated regional proportions of mixtures in the western Bering Sea collected during September-October, 2004. Standard deviation (SD) were calculated from 100 bootstrap resamplings of mixtures and baseline samples.

Station	Korea – Japan – Primory		Russia		Northwest Alaska		other North America	
	Estimate	SD	Estimate	SD	Estimate	SD	Estimate	SD
S1	0.3455	0.0363	0.5603	0.0443	0.0548	0.0173	0.0396	0.0123
S2	0.3479	0.0463	0.5461	0.0357	0.0822	0.0264	0.0238	0.0070
S3	0.2989	0.0352	0.4486	0.0290	0.2005	0.0306	0.0520	0.0046
S4	0.2558	0.0333	0.3755	0.0200	0.3022	0.0563	0.0665	0.0081
S5	0.3656	0.0323	0.5641	0.0460	0.0468	0.0176	0.0235	0.0062
S6	0.4973	0.0594	0.3479	0.0283	0.1271	0.0321	0.0272	0.0049
S7	0.4318	0.0436	0.3409	0.0346	0.2214	0.0267	0.0056	0.0026
S8	0.2890	0.0565	0.4442	0.0293	0.2053	0.0323	0.0615	0.0060
S9	0.3294	0.0354	0.5408	0.0409	0.1008	0.0240	0.0291	0.0039
S10	0.3580	0.0284	0.2480	0.0095	0.3074	0.0316	0.0864	0.0066
S11	0.3364	0.0254	0.4176	0.0286	0.1832	0.0327	0.0625	0.0091
S12	0.4568	0.0592	0.4993	0.0388	0.0336	0.0103	0.0105	0.0027
S13	0.3821	0.0399	0.5292	0.0430	0.0558	0.0208	0.0328	0.0125
S14	0.4076	0.0464	0.5494	0.0370	0.0311	0.0130	0.0118	0.0062
S15	0.5707	0.0372	0.3731	0.0270	0.0327	0.0147	0.0230	0.0064
S16	0.4625	0.0554	0.4594	0.0362	0.0716	0.0218	0.0063	0.0016
S17	0.6995	0.0445	0.2279	0.0170	0.0532	0.0075	0.0194	0.0018
S18	0.3977	0.0491	0.4866	0.0364	0.0848	0.0154	0.0305	0.0046
S19	0.3794	0.0428	0.4053	0.0307	0.0372	0.0135	0.1780	0.0302
S20	0.3524	0.0415	0.4440	0.0313	0.1887	0.0475	0.0151	0.0053
S21	0.5135	0.0395	0.4363	0.0235	0.0449	0.0174	0.0050	0.0023
S22	0.5552	0.0338	0.2681	0.0098	0.1325	0.0117	0.0442	0.0028
S23	0.6898	0.0527	0.2916	0.0127	0.0180	0.0071	0.0007	0.0004

DISCUSSION

1. Population structuring of chum salmon in the North Pacific

Both the phylogenetic relationship and the analyses of molecular variance model (AMOVA) provided geographic structures in the Pacific Rim chum salmon populations, where they were genetically differentiated among Japan, Russia, and North America (Sato *et al.,* 2004). The results from AMOVA revealed the very strong geographic structuring among three regions (Analysis Ⅰ, Table 2). However, sub-regional structure among six regional groups in Russia (Kamchtka, Sakhalin, Primorie, Magadan, Sernovodnaja, Amur) showed that it was obscure as a major group (AnalysisⅢ, Table 2). The estimates of probability test rejected the null hypothesis of no difference among six regional groups in Russia. In addition, the values from the percentage of variance and F-statistics didn't make it clear to combinate six regional groups into a major group. Furthermore, results of haplotype distribution and neighbor-joining distance tree, where some of the haplotypes were population-specific (Fig. 4; Appendix Ⅲ), showed that three populations (Avakumovka, Avakumovka (early-run), Narva river) in the primorie region and one population from sernovodnaja river genetically differed from other Russian populations (Fig. 5). In addition, they were similar to Korea-Japan

regional groups. In the previous study, after the recent retreat of glaciers and elevation of the ocean level the distribution of chum salmon affected genetic isolation between the southern region and the northern region (in press), and the divergence between the regions was observed (Brykov *et al.,* 2003).

2. Genetic Stock Identification (GSI) of mixtures in the Bering Sea and North Pacific Ocean.

It is widely accecpted that the distribution of chum salmon will be affected by temperature and the abundance of prey and the Bering Sea provides major feeding habitats for Pacific chum salmon as well as other salmonids species. According to this idea, the member nations of the NPAFC form the world's largest marine conservation area for salmonids under the Bering-Aleutian Salmon International Survey (BASIS) program. Those cooperative research activities revealed that Asian and North American salmon stocks are distributed in high density in the Bering Sea during summer. Especially, DNA analyses indicated that Asian stocks were dominant in the central Bering Sea (Winans *et al*., 1998; Wilmot *et al*., 1998; Urawa *et al*., 2004; Sato *et al*., 2004; Yoon *et al*., 2005 (NPAFC 2004)).

In late summer 2004 the ratios of mixing stocks dominated by two stocks, Korean-Japanese-Primorie and Russian chum salmon in the western Bering Sea

were approximately 42% JKP stock, 41% Russian stock, 17% NA stocks (actually 13% Northwest Alaskan stocks, and 4% other North American stocks). Similarly, the approximate profile for Japanese/Russian/North American stocks in the central Bering Sea was 45:43:13% in September 2002 (Yoon *et al.,* 2004). In September 2003, the approximate profile for Japanese/Russian/North American stocks in the eastern Bering Sea was 45:30:25%, which showed the proportion of Russian stocks was decreased. On the other hand, the profile of North American stocks was increased to the eastern parts in the Bering Sea. Moreover, in September 2003 this trend was apparent in the Gulf of Alaska, where the stock profile of mixtures was estimated at primarily 52% North American stocks, 27% Russian stocks, and 21.6% Japanes stocks (Fig. 8).

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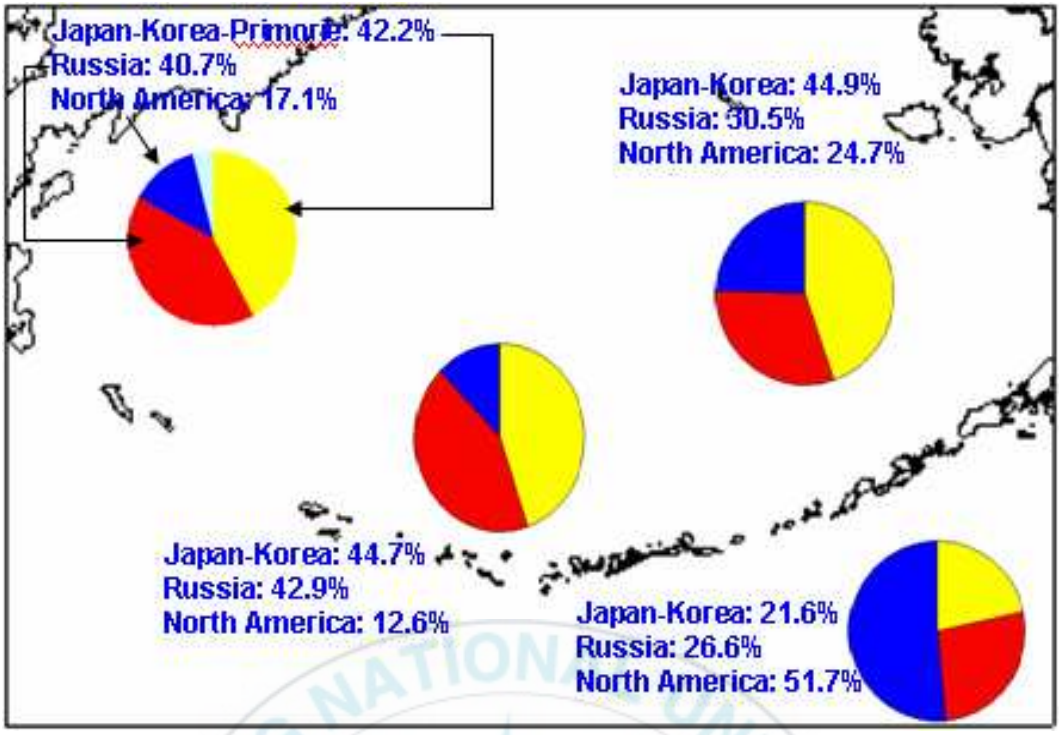

Fig. 8. GSI-estimated stock composition of chum salmon mixtures in the Bering Sea and the Gulf of Alaska during 2002-2004 (cited and modified from Yoon *et al.,* 2004).

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APPENDIX Ⅰ. Sampling locations, date of collection, the numbers of chum salmon samples (N) used for mtDNA analysis.

	Sampling location	Date of collection	N
1	Chitose River*	14 Oct. 1996	51
2	Tokushibetsu River*	23 Sept. 1997	51
3	Tokoro River (late run)	20 Nov. 1998	44
4	Tokoro River (early run)*	13 Oct. 1999	49
5	Nishibetsu River*	25 Sept. 1997	41
6	Kushiro River*	22 Oct. 1998	49
7	Tokachi River*	17 Oct. 1996	46
8	Yurappu River*	17 Nov. 1998	40
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, Nigata Pref.*	23-24 Oct. 1996	49
16	Jintsu River, Toyama Pref.*	7 Nov. 1995	49
17	Namdae River	13 Nov. 2000	46
18	Anadyr River*	1990	43
19	Anadyr River (early run)	2000	33
20	Hairsova River*	1993	41
21	Kamchatka River*	1991	46
22	Kamchatka River (early run)		50
23	Vorovskaya River*	1990	32
24	Kol River*	1991	44
25	Pymta River	2003	49

APPENDIX Ⅰ. (continued)

Sampling location		Date of collection	N
26	Utka River	2002	20
27	Apuka River	2002	50
28	Olutolsky Bay	2002	50
29	Tigil River	2002	44
30	Bolchaya River	1999	50
31	Bolchaya Malki River	2001	50
32	Kalininka River*	1994	42
33	Kalininka River (early run)	2003	25
34	Belaya River	2003	25
35	Ada Tymovo River	2003	25
36	Taranay River	2003	25
37	Naiba River	1995	16
38	Okhotskoe River	2003	25
39	Avakumovka River*	1994	30
40	Avakumovka River (early run)	2001	26
41	Narva River	1995	34
42	Ola River*	1999	33
43	Magadan River*	1990	37
44	Magadan River (early run)	1991	42
45	Taui	1999	39
46	Sernovodnaja River	1995	33
47	Amur River*	9 Sept. 2000	50
48	Salmon River*	1991	45
49	Sheenjek River (fall run)*	1992	45
50	Andreafsky River (summer run)*	1993	48
51	Togiak River*	1993	49
52	Noatak	1991	50
53	Tanana River	1993	50

APPENDIX Ⅰ. (continued)

Sampling location		Date of collection	N
54	Unalakleet	1992	50
55	Kwethluk River	1994	50
56	Upper Nushagak	1993	50
57	Kizhuyak River*	1992	46
58	Olsen Creek*	1992	45
59	Kitoi Hatchery	1993	50
60	McNeil River	1994	50
61	Belkofski River*	1992	44
62	Chiginagak	1991	50
63	Sawmill Creek, Berner's Bay*	28 July. 1993	50
64	Long Bay, Chichigof Island*	25-26 Aug. 1991	49
65	Whale Bay, Baranof Island*	12 Aug. 1993	48
66	Port Beauclerc, Kuiu Island*	20 Aug. 1995	45
67	Fish Creek, Portland Canal*	25 Sept. 1988	49
68	Disappearance Creek, POW Island*	25 Sept. 1998	50
69	Ecstall River, Skeena River area*	12 Sept. 1988	45
70	Bag Harbor, QCI*	mid-Oct. 1989	50
71	Nekite Channel*	15 Sept. 1989	33
72	Nooksack River*	1998	47
73	Quilcene Bay*	1998	49
74	Blackjack Creek*	1998	50
75	Satsop River*	1998	49
76	Hamilton Creek*	1998	43

* Cited from Sato *et al.,* (2004)

APPENDIX Ⅱ. Variable nucleotide position in the 5' half of mtDNA control region of chum salmon collected from 76 populations in a total of 3,266 individuals (Sato *et al.*,, 2004).

Haplotype	10	50	42	57	70	78	96	108	154	194	231	242	250	260	339	340	386
A-1	T	T	A	A	T	T	-	A	C	A	T	C	T	A	T	C	G
A-2	C	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
A-3	.	.	G	.	.	.	-	.	.	.	.	.	.	.	.	.	.
A-4	.	.	.	.	.	.	-	C	.	.	.	.	.	.	.	.	.
A-5	.	.	.	.	.	.	-	.	.	T	.	.	.	.	.	.	.
A-6	.	.	.	.	.	.	-	.	.	.	C	.	.	.	.	.	.
A-7	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
A-8	.	.	.	.	.	.	A	.	.	.	.	.	.	G	.	.	.
B-1	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	-
B-2	.	C	.	.	.	.	-	.	.	.	.	.	.	.	.	.	-
B-3	.	.	.	.	.	.	-	.	G	.	.	.	.	.	.	.	-
B-4	.	.	.	.	.	.	-	.	.	.	C	.	.	.	.	.	-
B-5	C	.	.	.	.	.	-	.	G	.	.	.	.	.	.	.	-
B-6	.	.	.	.	C	.	-	.	G	.	.	.	.	.	.	.	-
B-7	.	.	.	.	.	C	-	.	G	.	.	.	.	.	.	.	-
B-8	.	.	.	.	.	.	-	C	G	.	.	.	.	.	.	.	-
B-9	.	.	.	.	.	.	-	.	G	.	C	.	.	.	.	.	-
B-10	.	.	.	.	.	.	-	.	G	.	.	T	.	.	.	.	-
B-11	.	.	.	.	.	.	-	.	G	.	.	.	C	.	.	.	-
B-12	.	.	.	.	.	.	-	.	G	.	.	.	.	G	.	.	-
B-13	.	.	.	.	.	.	-	.	G	.	.	.	.	.	A	.	-
B-14	.	.	.	.	.	.	-	.	G	.	.	.	.	.	.	.	-
B-15	.	.	.	.	.	.	-	.	G	.	.	.	.	.	.	.	-
B-16	.	.	.	.	.	.	-	.	G	.	.	.	.	.	A	.	-
B-17	.	.	.	.	.	.	-	.	G	.	.	.	.	.	A	.	-
C-1	.	C	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.
C-2	.	C	.	T	.	.	-	.	.	.	.	.	.	.	.	.	.
C-3	.	C	.	.	C	.	-	.	.	.	.	.	.	.	.	.	.
C-4	.	C	.	.	.	.	-	T	.	.	.	.	.	.	.	.	.
C-5	.	C	.	.	.	.	-	.	.	.	C	.	.	.	.	.	.

APPENDIX Ⅲ. Distribution of mtDNA control region haplotypes among 76 populations of chum salmon in the Pacific Rim.

POP. Number	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
1	22				1						14	2													
2	30										13														
3	26						1				8														
4	21					1			1		16														
5	12										18														
6	23	1	4						1		8														
7	18					2		4			12														
8	24										6														
9	25																								

10	20		4	1	7	
11	26		2			
12	24		1		1	
13	19				5	
14	26				4	1
15	29	2			8	
16	37				2	
17	36				6	

APPENDIX Ⅲ. (continued)

	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
18											35														
19											30														
20											32						1								
21											31				1										
22											48														
23											38						1			1					
24											38						2			1					
25											40						1								
26											18														
27											44											3			
28											44														
29											37						3								
30											48														
31											49														
32											20						16								
33											14					6	4								
34	1										17					2	3								
35											22														

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APPENDIX Ⅲ. (continued)

	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
36									1		19					1	3								
37									1		11					1	1								
38											19					4	2								
39	7										9					1									
40	4										7					1									
41	8										6														
42									1		20			1			3								
43										4	31						1								
44											32						1								
45									1		27														
46	3					1					26														
47									2		45						2								
48											48														
49											45														
50											45														
51											48												1		
52											44						5			1					
53										1	49														

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APPENDIX Ⅲ. (continued)

	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
54											50														
55											50														
56											49														
57											36						1				6				
58											35								2		6				2
59											49														
60											49														
61											37										5				
62											47										3				
63											39						1		5		5				
64											40						1		1		7				
65											33						2				13				
66											40						4				1				
67											45										3				1
68											33						5				12				
69											29						1				15				
70											32								1		17				
71											25										8				

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APPENDIX Ⅲ. (continued)

	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
72											39										8				
73											41							3			5				
74											45										3			2	
75											23						1				17	8			
76											23		6								12	2			

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