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Syuiti ABE^{1, 2}, Shunpei SATO³, Hiroyuki KOJIMA⁴, Junko ANDO³, Hironori ANDO³, Richard L. WILMOT⁵, Lisa W. SEEB⁶, Vladimir EFREMOV⁷, Larry LECLAIR⁸, Wally BUCHHOLZ⁹, Deuk-Hee JIN¹⁰, Shigehiko URAWA¹¹, Masahide KAERIYAMA⁴, and Akihisa URANO^{3, 12}

¹*Laboratory of Animal Cytogenetics, Center for Advanced Science and Technology, Hokkaido University, Sapporo, 060-0810, Japan*

²*Laboratry of Cytogenesis, Gradate School of Environmental Earth Science, Hokkaido University, Sapporo, 060-0810, Japan*

³*Division of Biological Science, Graduate School of Science, Hokkaido University, Sapporo, 060-0810, Japan*

⁴*Graduate School of Science and Engineering, Hokkaido Tokai University, Sapporo, 005-8601, Japan*

⁵*Auke Bay Laboratory, Alaska Fisheries Science Center, NOAA, Juneau, AK, U.S.A.*

⁶*Alaska Department of Fish and Game, Anchorage, AK, U.S.A.*

⁷*Russian Academy of Science, Vladivostok, Russia*

⁸*Washington State Department of Fish and Wildlife, Washington, U.S.A.*

⁹*U.S. Fish and Wildlife Service, Anchorage, AK, U.S.A.*

¹⁰*Kangnung National University, Kangnung, Korea*

¹¹*National Salmon Resources Center, Sapporo, 062-0922, Japan*

¹²*Field Science Center, Hokkaido University, Sapporo, 060-0811, Japan*

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Genetic differentiation among Pacific Rim populations of chum salmon inferred from mitochondrial DNA sequence variation

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¹Laboratory of Animal Cytogenetics, Center for Advanced Science and Technology, Hokkaido University, Sapporo, 060-0810, Japan

²Laboratory of Cytogenesis, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo, 060-0810, Japan

³Division of Biological Science, Graduate School of Science, Hokkaido University, Sapporo, 060-0810, Japan

⁴Graduate School of Science and Engineering, Hokkaido Tokai University, Sapporo, 005-8601, Japan

⁵Auke Bay Laboratory, Alaska Fisheries Science Center, NOAA, Juneau, AK, U.S.A.

⁶Alaska Department of Fish and Game, Anchorage, AK, U.S.A.

⁷Russian Academy of Science, Vladivostok, Russia

⁸Washington State Department of Fish and Wildlife, Washington, U.S.A.

⁹U.S. Fish and Wildlife Service, Anchorage, AK, U.S.A.

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¹¹National Salmon Resources Center, Sapporo, 062-0922, Japan

¹²Field Science Center, Hokkaido University, Sapporo, 060-0811, Japan

ABSTRACT

We examined genetic differentiation among Pacific Rim populations of chum salmon (*Oncorhynchus keta*) by mitochondrial (mt) DNA analysis. Nucleotide sequence analysis of about 500 bp variable portion in the 5' end of the mtDNA control region revealed 20 variable nucleotide sites, defining 30 haplotypes of three genealogical groups (A, B, and C), in more than 2100 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 the regions, in that A and C group haplotypes characterized Asian populations and B group 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 AMOVA and contingency χ^2 test inferred strong structuring among the three geographic groups of populations and weak to moderate structuring within Japanese and North American populations. Pairwise population F_{ST} estimates increased with distance, which were largest between Japan and North America, followed by estimates between Japan and Russia and between Russia and North America, although the estimates within regions were not necessarily parallel with geographic distance. These findings suggested that the observed geographic pattern might be influenced primarily by range expansions or colonizations and secondarily by low or restricted gene flow between local groups within regions. In addition to the analysis of population structure, the present mtDNA data would become useful for constructing a baseline for stock identification of mixed high seas populations of chum salmon.

Key words: mitochondrial DNA, chum salmon, genetic differentiation, population structure

INTRODUCTION

Chum salmon (*Oncorhynchus keta*) have the widest natural geographical distribution among all Pacific salmon species in the Pacific Rim, ranging from Korea and Japan northward to the Arctic coasts of Russia and North America and southward to Oregon (Salo 1991). Spawning adults, like other Pacific salmon, are anadromous to their natal rivers. Such restricted homing behavior can lead geographically distinct populations to partial genetic isolation. Estimation of genetic variation among or within Pacific Rim populations of chum salmon is therefore important when addressing population history, the mechanisms of ocean migration and the stock composition of high seas mixed aggregations from commercial fisheries.

The genetic variation of chum salmon has so far been examined by allozyme analysis (Winans et al. 1994, Wilmot et al. 1998); however, stock structure resolution remains mostly at the regional- or continental-levels (Brown et al. 1979). Moreover, allozyme data are often inadequate for discriminating the causal factors of population divergence (Zhivotovsky et al. 1994).

Recently developed molecular techniques are expected to provide a powerful means with an increased accuracy and resolution to observe genetic variation in salmon populations (Ferguson et al. 1995). Maternally inherited mitochondrial (mt) DNA has higher sequence variability than single copy nuclear genes (Brown et al. 1979). In particular, the control region is considered to be the most variable portion in mtDNA, with two to five times higher rates of nucleotide substitution than protein-coding regions (Moritz et al. 1987, Meyer 1993). Thus, analyses of mtDNA may, at present, be the method of choice for phylogenetic, population genetic, and evolutionary studies (Moritz et al. 1987).

Previous studies on restricted fragment length polymorphisms (RFLPs) showed low levels of variation in mtDNA sequences of salmonids including chum salmon (Cronin et al. 1993, Bickham et al. 1995). Moreover, comparative analyses of allozyme and mtDNA RFLP data produced similar results when used for stock identification of mixed-stock chum salmon fisheries (Seeb & Crane 1999b). Recently, (Sato et al. 2001) detected greater variation in the mtDNA control region by nucleotide sequence analysis than the variation observed by the previous RFLP analyses (Park et al. 1993). This finding suggests an increased potential for mtDNA sequence analysis to estimate the genetic variation of chum salmon populations.

In the present collaborative study, nucleotide sequencing of the mtDNA control region was conducted to examine its potential for detecting genetic variation and resolving the 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 1617 individuals of 36 populations from Japan (4 populations), Korea (one population), Russia (10 populations), and North America including Alaska (13 populations), British Columbia (three populations), and Washington (five populations) between 1988 and 2000 (Table 1 and Figure 1). The fish used in the present study were captured when they returned to their natal river to spawn. Most samples were previously used

for allozyme and/or mtDNA RFLP analyses (Kondzela et al. 1994, Wilmot et al. 1994, Winans et al. 1994, Seeb and Crane 1999b). Although the sampling year was different for the fish populations used in the present study, we assumed that the genetic composition was temporally stable within the same sampling locales, as has been suggested by the previous allozyme studies (Seeb and Crane 1999b, Wilmot et al. 1994). A possible genetic difference, if any, between summer- and fall-run fish was examined for those collected from the Yukon River. Early- and late-run populations from two Japanese rivers also were included in the analysis as in {Sato, Ando, et al. 2001 14293 /id}. The liver and blood samples were stored at -80? and muscle samples were stored in 100% ethanol at room temperature until DNA extraction.

DNA extraction

DNA was isolated from stored tissue and blood samples following the routine phenol-chloroform extraction method of (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, 10mM Tris-HCl, and 1 mM EDTA, pH8.0). The frozen liver samples were immediately homogenized in the same solution. In brief, about 50 µl of whole blood and homogenates of liver or muscle tissue were added to 500 µl STE buffer containing 500 µg/ml proteinase K and 0.5% SDS, and incubated at 37? overnight. DNA was extracted with a mixture of phenol (250 µl) and 24:1 chloroform:isoamylalcohol (250 µl) three times, 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 conditions by which the control region of mtDNA was amplified and the PCR primers used were as described in {Sato, Ando, et al. 2001 14293 /id}. The PCR products were purified by the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) after confirmation of their sizes by gel-electrophoresis, and subjected to the direct nucleotide sequence analysis of about 500 from the 5' end of the mtDNA control region with a Hitachi SQ-5500L DNA Sequencer (Hitachi, Tokyo) {Sato, Ando, et al. 2001 14293 /id}.

Nucleotide sequence data analysis

For data analysis, the nucleotide sequences of 537 previously examined individuals from 12 Japanese populations were also included (Sato et al. 2001). Thus, a total of 2154 individuals from 48 Pacific Rim populations were analyzed in the present study (Table 1 and Figure 1). Haplotype variation was determined by aligning sequence data from the 5' end of the mtDNA control region using GENETIX-WIN version 4.0.6 (Software Development Co., Ltd, Tokyo). A parsimony network which connects the observed haplotypes was drawn using two different programs; the Network version 3.1012 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) to estimate the genealogical relationship among the observed haplotypes.

Population genetic data analysis

Haplotype diversity was calculated for each population according to {Nei 1973 145 /id}. Nucleotide diversity was estimated for each population according to (Nei and Tajima 1981). The heterogeneity of the haplotype frequencies within and between geographic regions was evaluated

using the contingency χ^2 test {Roff & Bentzen 1989 14276 /id}, with 1,000 Monte Carlo simulations using the CHIRXC program {Zaykin & Pudovkin 1993 14335 /id}. In order to assess the extent of genetic differentiation at the different level of geographic hierarchy, the overall molecular variance was partitioned into components corresponding to the population divergence within and among regions using the analysis of molecular variance model (AMOVA) of {Excoffier, Smouse, et al. 1992 14273 /id}. For AMOVA, populations were grouped geographically by the neighbor-joining method {Saitou & Nei 1987 77 /id}, in which the obtained-topology was tested by a bootstrap analysis with 1,000 pseudo-replicate trees. Pairwise F_{ST} values were calculated to estimate the genetic distance between populations according to {Slatkin 1995 14337 /id}. Significance of the variance components and F_{ST} values was tested with a permutation method.

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 revealed 20 variable sites in a total of 2154 individuals from 48 populations, which defined a total of 30 haplotypes (A-1 to C-5) as shown in Table 2. The observed nucleotide sequence variations included one nucleotide insertion, one nucleotide deletion, and 18 nucleotide substitutions including 11 transitions and seven transversions (see Table 2). Accordingly, designation of the 12 haplotypes reported in the previous study ({Sato, Ando, et al. 2001 14293 /id}) was changed as follows: OKDL-1 as A-1, OKDL-2 as A-5, OKDL-3 as A-6, OKDL-4 as A-7, OKDL-5 as A-8, OKDL-6 as B-1, OKDL-7 as B-3, OKDL-8 as B-4, OKDL-9 as C-1, OKDL-10 as C-2, OKDL-11 as C-4, and OKDL-12 as C-5. The sequences of 18 newly identified mtDNA control region haplotypes were registered in the DDBJ/EMBL/GenBank with accession numbers ***** to *****.

Haplotype genealogy. 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, as presented in Figure 2. The observed haplotypes of chum salmon could be 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. A-1, B-3, and C-1 were focal haplotypes in the respective clades. The T to C transition at nucleotide 30 separated clade C from clade A, and a deletion at nucleotide 386 and a C to A substitution at nucleotide 395 discriminated clade B from clades A and C, respectively.

Haplotype distribution in Pacific Rim populations

The distribution of 30 haplotypes among 48 populations of chum salmon is presented in Table 3. The occurrence of haplotypes in the Pacific Rim was nonrandom and mostly associated with the regions.

Asian populations. The highest number of haplotypes (16) among the regions examined was found in 16 Japanese populations, where all three haplotype clades occurred, except for the Tsugaruishi late-run and the Otsuchi population without clade B haplotypes (see Table 3 and Fig. 1). The A-1 and C-1 haplotypes were common in all the populations of Japan. The B-3 was another common haplotype in the Japanese populations except for the above two (Table 3). Thus, the frequency and composition of the haplotypes were different in early- and late- runs from the Tsugaruishi River in the Pacific coast of Honshu, whereas the Tokoro River early and late runs in Hokkaido showed essentially similar haplotype distribution (Sato et al. 2001).

Thirteen and four haplotypes occurred in 10 Russian populations and one Korean population, respectively (Table 3). The occurrence of all three haplotype clades was also observed in one population each from the Namadae River in Korea and the Avakumovka River in Russia, both of which are on the Sea of Japan coast (see Table 3 and Figure 1). Nine other Russian populations showed haplotypes from clades B and C, although the latter was less frequent in number than the former (Table 3). In particular, the haplotype B-3 was common and predominant in most of the Russian populations (Table 3).

North American populations. On the other hand, 21 North American populations exhibited 10 clade B and one clade C haplotypes (Table 3). The latter was rare (<8%) and occurred only in one population each from the Alaska Peninsula (Belkofski River) and Kodiak Island (Kizhuyak River) (see Table 3 and Figure 1). Among the observed clade B haplotypes, the B-3 and B-13 were common in most of the North American populations, although the latter was less frequent in number than the former. In particular, four populations in northwest Alaska, i.e. the Salmon River, Sheenjak River (summer-run) and Andreafsky River (fall-run), both tributaries of the Yukon River, and the Togiak River, were missing the B-13 haplotype and were fixed or almost fixed for the B-3 haplotype (see Table 3 and Figure 1). Other North American populations usually added one or two haplotypes to the B-3 and B-13. Therefore, the occurrence of haplotypes in each population apparently decreased in number in North America when compared with Japan and Russia.

Inter-continental haplotype distribution. Among the observed haplotypes, the region-specific haplotypes were 10 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, eight clade B (B-10 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 were common among the three geographic groups of populations, although the latter was quite rare in frequency and its occurrence was limited in the North American populations (the Belkofski River in Alaska Peninsula and the Kizhuyak River in Kodiak Island). Besides these haplotypes, the A-1, B-1, C-2, and C-5 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 may be safe to state that the clade A and C haplotypes characterize the Asian populations, and that the clade B haplotypes distinguish 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, as shown in Table 4. These findings suggest a greater genetic variation in the populations of Japan than those of Russia and North America.

Population cluster. The examined populations were clustered using the neighbor-joining method (Figure 3). The population consensus tree definitely separated Japan/Korea from the rest of the geographic groups including Russia and North America with high bootstrap support (100%), although one Russian population (Avakumovka) on the Sea of Japan coast was included in the Japan/Korea cluster. Interestingly, northwest Alaska was separated from the rest of North American groups with more than 50% of the bootstrap replicates, but contained two Russian populations (the Kamchatka River in eastern Kamchatka Peninsula and the Kalininka River in Sakhalin Island) within the same cluster. Other Russian populations formed a separate cluster with more than 95% bootstrap support. Thus, four population clusters of Japan/Korea, Russia, northwest Alaska, and the rest of 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, those in Japan, Russia, and North America. Such a significant heterogeneity ($p < 0.001$) was also observed for the set of populations from Japan and Russia, Japan and North America, and Russia and North America. Furthermore, significant heterogeneity was observed for the set of populations within North America ($p < 0.005$), suggesting five regional groups of northwest Alaska, Alaska Peninsula/central Alaska, southeast Alaska, British Columbia, and Washington. Similar levels of heterogeneity ($p < 0.005$) were also apparent among the Hokkaido, Pacific coast of Honshu, and the Sea of Japan coast populations (Sato et al. 2001). No significant heterogeneity was shown for the populations in the Yukon summer- and fall-runs from northwest Alaska and the late- and early-runs from the Tokoro River in Hokkaido (Sato et al. 2001), whereas significant heterogeneity was observed between the Tsugaru late- and early-runs from the Pacific coast of Honshu ($p < 0.01$).

Geographic hierarchy in the Pacific Rim populations. Partitioning of molecular variance with a set of AMOVAs (Table 5) revealed the following population structure in chum salmon: (i) very strong geographic structuring among Japan, Russia, and North America (56.2% of the variance, Analysis I), as compared with the average extent of structuring among populations within each geographic group (4.3% of the variance); (ii) weak to moderate structuring among northwest Alaska, Alaska Peninsula/central 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 regional groups (Tables 1 and 3) (18.2% of the variance, $p > 0.05$, Analysis III) and very weak structuring among local populations within groups in Russia (3.0% of the variance). This may be the results of insufficient sampling.

Genetic differentiation among Pacific Rim populations. As shown in Table 6 the pairwise population F_{ST} values were generally higher among the geographic groups between continents than within continents. The F_{ST} estimates were greater between Japan and North America (0.337 to 0.930) than between Japan and Russia (0.008 to 0.990) or between Russia and North America (0.000 to 0.636). These results suggest little gene flow or very strong genetic differentiation among the three geographic groups of populations, and the greatest differentiation between Japan and North America. The F_{ST} estimates also suggested a certain level of genetic differentiation between Hokkaido and Honshu in Japan (~ 0 to 0.328) and between northwest Alaska and the rest of North American groups (0.004 to 0.306). In the latter, however, the F_{ST} estimates did not always seem parallel with distance (Table 6).

DISCUSSION

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

Our nucleotide sequence analysis detected a similar or even higher level of variation in the

5' end of the control regions in other *Oncorhynchus* species including *O. mykiss*, *O. kisutch*, and *O. tshawytscha* (Nielsen et al. 1994). Such an inter-specific comparison must be interpreted carefully, since the extent of sequence variation in the control region is site-specific and species-specific in fish (Meyer 1993). However, the variations found herein were apparently higher than those detected in the previous RFLP analysis of chum salmon mtDNA in terms of the number of observed haplotypes (Cronin et al. 1993, Seeb and Crane 1999a). The present findings indicate an increased potential for mtDNA sequence analysis to estimate the genetic variation within populations and to examine the structure among population of chum salmon.

The observed 30 haplotypes were genealogically connected in the three clades of A, B and C (Figure 2). The A-1, B-3 and C-1 haplotypes are presumably ancestral within the clades A, B and C, respectively, since the three haplotypes are more abundant and central in each genealogy. However, the relationships among the three clades are ambiguous, as shown in Figure 2. The three star-like genealogies also suggest that most of the infrequent or rarer haplotypes radiating from the central ones might have evolved after colonization of chum salmon in each of the three geographic regions. This would be further advocated by the findings that the radiated haplotypes include most of the region-specific haplotypes. The Japanese populations have some features not found in other geographic groups, such as the largest number of haplotypes including the region-specific ones, the greatest haplotype diversity, and the presence of all three haplotype genealogies detected herein. These findings suggest that Japanese populations may be older than Russian and North American populations.

The present AMOVAs and contingency χ^2 test revealed a clear geographic structuring in the Pacific Rim chum salmon populations, showing distinct genetic differentiation among Japan, Russia and North America. This result was corroborated further by the pairwise population F_{ST} estimates, in that the estimates increased with distance, suggesting genetic differentiation by range expansions or colonizations. Genetic differentiation among the three regional groups of chum salmon was also suggested by the previous studies using variation of allozyme loci (Okazaki 1983), Wilmot et al. 1998, (Seeb and Crane 1999b), mtDNA RFLPs (Seeb and Crane 1999a), and minisatellite DNA (Taylor et al. 1994). However, only a few studies have inferred geographic structuring within regions (Phelps et al. 1994, Wilmot et al. 1998, (Seeb and Crane 1999b). In the present study, such a subregional structuring was suggested within the Japanese and North American populations, although the extent of structuring within regions was weak to moderate as compared with inter-continental structuring (Table 5). In Japan, higher F_{ST} estimates between Hokkaido and Honshu populations than within Hokkaido or Honshu populations imply low or restricted gene flow between Hokkaido and Honshu. As has been suggested previously, such low gene flow between the two regions in Japan may be ascribed to the differences in the route of spawning migration, run timing, and distance among pairwise populations, in addition to possible geological or historical factors (Sato et al. 2001), although contemporary forces such as human-mediated extensive transplantation of stocks may not be excluded in the observed population structure. For instance, the Hokkaido and Honshu populations have undergone extensive hatchery domestication and transplantation of eggs and fry from one river population to another was historically common (Kijima and Fujio, 1982). In the present study, however, such a contemporary influence should have been minimized by sampling late-run fish, particularly in Honshu, since introduced Hokkaido populations migrate earlier than native Honshu populations (Salo 1991).

The moderate structuring within North America, i.e. northwest Alaska, Alaska Peninsula/central Alaska, southeast Alaska, British Columbia, and Washington, is another intriguing finding. Similar, if not the same, structuring was also suggested by the previous allozyme studies

(Seeb and Crane 1999b). The present contingency χ^2 test and pairwise population F_{ST} estimates imply low gene flow among these five regions, particularly between northwest Alaska and the rest of the geographic groups (Table 6). Although the factors involved in the observed structuring within North America remain mostly unknown, the glacial history is likely to influence the population structure of North American salmonids (Utter et al. 1980, Gharrett et al. 1983, 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 northwestern Alaska and Russia were recolonized from Beringia, the populations of the Gulf of Alaska, British Columbia and Washington were recolonized from Cascadia (Seeb & Crane 1999a). The inference of geographic grouping of North American chum salmon populations from the present mtDNA analysis may reflect this glacial history, although this inference should be confirmed by further studies with sufficiently detailed sampling, using other competent methods to estimate the population history, such as the nested cladistic analysis (Templeton et al. 1992). As well, more extensive sampling of Russian populations is needed to refine the geographic structuring, since the AMOVA failed to demonstrate higher level of geographic hierarchy (Table 5).

Distinct genetic population structure in the Pacific Rim chum salmon revealed herein also suggests that mtDNA sequence analysis may provide better estimation of stock composition in mixed ocean populations. In this context, the region-specific haplotypes, though infrequent, will be available for direct allocation of fish to the region of origin. Again, we need more populations from Russia and North America in order to establish the mtDNA baseline necessary for accurate stock identification of mixed chum salmon aggregations in ocean. In addition, as mentioned above, our mtDNA sequence data may become 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.

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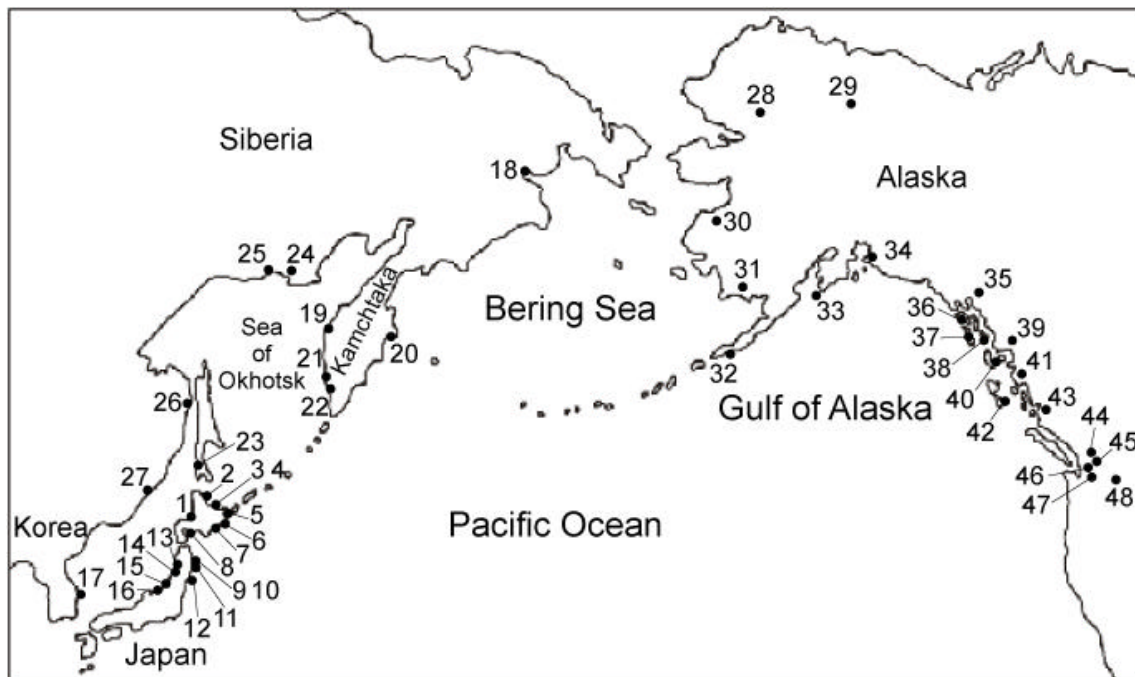


Figure 1. Geographical position of sampling site (see Table 1 for the site names).

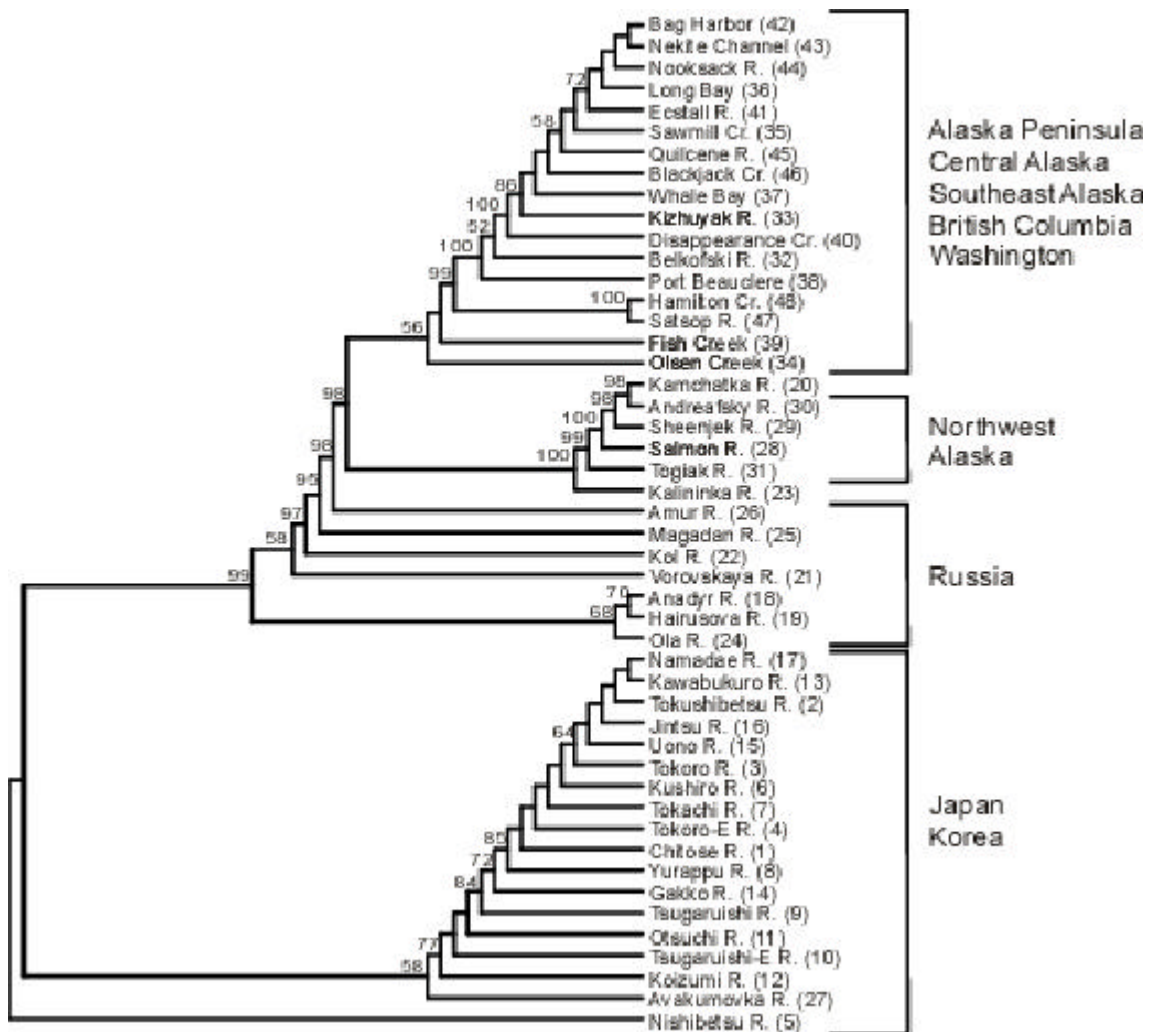


Figure 3. Phenogram of 48 chum salmon collections based on the haplotype frequencies and average nucleotide diversities between collections by neighbor-joining method (Saitou & Nei 1987). Nodal numbers in the phenogram are bootstrap values based on 1,000 replications.

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

Sampling location	Date of collection	N**
Japan		
<i>Hokkaido island</i>		
1 Chitose River*	14 Oct. 1996	51
2 Tokushibetsu River*	23 Sep. 1997	51
3 Tokoro River*	20 Nov. 1998	44
4 Tokoro River*	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
<i>Honshu island</i>		
9 Tsugaruishi River, Iwate Pref. *	10 Dec. 1997	44
10 Tsugaruishi River, 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

Korea

17 Namadae River

13 Nov. 2000

46

Sampling location	Date of collection	N*
Russia		
<i>Anadyr</i>		
18 Anadyr River	1990	43
<i>Kamchatka Peninsula</i>		
19 Hairsova River	1993	41
20 Kamchatka River	1991	46
21 Vorovskaya River	1990	32
22 Kol River	1991	44
<i>Sakhalin island</i>		
23 Kalininka River	1994	42
<i>Magadan</i>		
24 Ola River	1990	33
25 Magadan River	1991	37
<i>Nikorayevsk na Amur</i>		
26 Amur River	9 Sep. 2000	50
<i>Primorye</i>		
27 Avakumovka River	1994	30
North America		
<i>Northwest Alaska</i>		
28 Salmon River	1991	45
29 Sheenjek River	1992	45

30	Andreafsky River	1993	48
31	Togiak River	1993	49
Sampling location			Date of collection
N*			
<i>Alaska Peninsula</i>			
32	Belkofski River	1992	44
<i>Central Alaska</i>			
33	Kizhuyak River	1992	46
34	Olsen Creek	1992	45
<i>Southeast Alaska</i>			
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
<i>British Columbia</i>			
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
<i>Washington</i>			
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).

**N is the number of samples.

Table 2. Variable nucleotide positions from the 5' half of the 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	G	-	A	.	.	.
B-8	C	G	-	A	.	.	.
B-9	G	.	C	-	A	.	.	.

Haplotype	10	30	42	57	70	78	96	108	154	194	231	242	250	260	339	340	386	395	401	471
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 hyphens represent deletions and the dots represent the same nucleotide at the same position as in A-1.

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																														
<i>Hokkaido island</i>																														
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
<i>Honshu island</i>																														
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

	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	
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																															
<i>Anadyr</i>																															
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	
<i>Kamchatka Peninsula</i>																															
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	
<i>Sakhalin island</i>																															
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	
<i>Magadan</i>																															
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	
<i>Nikorayevsk na Amur</i>																															
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	

	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	
<i>Primorye</i>																															
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	0	6	0	0	0	7
North America																															
<i>Northwest Alaska</i>																															
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	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	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	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	0
<i>Alaska Peninsula</i>																															
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	
<i>Central Alaska</i>																															
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	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	0
<i>Southeast Alaska</i>																															
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	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	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	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	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	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	0

	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	
<i>British Columbia</i>																															
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	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	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	0
<i>Washington</i>																															
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	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	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	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	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	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	

Table 4. Haplotype diversity (h , \pm SD) and nucleotide diversity (π , in parenthesis) within 48

populations calculated from mtDNA haplotype frequencies.

Population	h (?)	Population	h (?)	Population	h (?)
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 Channell	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)	Sheenjok	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)		

Table 5. Results of the hierarchical analyses of molecular variance for chum salmon. The percentage of variance (%), probability estimated from permutation (P), and the F-statistics (F) are given at each hierarchical level (Exoffier et al. 1992).

Variance component	%	P	F
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	>0.05	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

Table 6. Pairwise F_{ST} estimates for regional chum salmon populations excluding one Korean population by mtDNA sequence analysis. Maximum (top) and minimum (bottom) F_{ST} values within and between regions are shown.

Region	Japan		Russia		North America			
	HOK	HON	RUS	NWA	AP/ CLA	SEA	BCL	WSG
Japan								
Hokkaido (8)	0.121							
	-0.019							
Honshu (8)	0.328	0.125						
	-0.026	-0.026						
Russia								
Russia (10)	0.752	0.990	0.566					
	0.008	0.048	-0.023					
North America								
Northwest Alaska (4)	0.807	0.930	0.636	0.000				
	0.527	0.757	0.011	-0.002				
Alaska Peninsula/ Central Alaska (3)	0.737	0.868	0.566	0.118	0.036			
	0.337	0.613	0.000	0.063	-0.005			
Southeast Alaska (6)	0.779	0.904	0.610	0.224	0.077	0.138		
	0.475	0.703	0.008	0.004	-0.015	-0.012		
British Columbia (3)	0.745	0.878	0.585	0.306	0.096	0.219	-0.003	
	0.458	0.700	0.110	0.237	-0.008	-0.020	-0.020	
Washington (5)	0.780	0.904	0.612	0.261	0.094	0.179	0.248	0.211
	0.451	0.682	0.017	0.013	-0.012	-0.016	-0.010	0.007

