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Genetic Variation among Japanese Populations of Chum Salmon Inferred from the Nucleotide Sequences of the Mitochondrial DNA Control Region

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ABSTRACT—We examined the nucleotide sequences of 500 bp variable portion from the 5' end of mitochondrial (mt) DNA control region in about 500 individuals from 12 populations that were captured in 11 rivers, six in Hokkaido and five in Honshu, Japan. Comparison of the sequences showed 10 variable sites, defining a total of 12 haplotypes in the examined individuals. All the 12 haplotypes occurred in seven Hokkaido populations, whereas only six haplotypes were found in the five Honshu populations. Among these haplotypes, two were common in all the Hokkaido and Honshu populations. The AMOVA analysis inferred a genetic differentiation among three geographic regions, i.e. Hokkaido, Pacific Ocean coast in Honshu, and Japan Sea coast in Honshu. Haplotype diversity was higher in the populations of Hokkaido than those of Honshu, indicating a greater genetic variation in the Hokkaido than the Honshu populations. The estimates of pairwise population F_{ST} suggested that the regional differentiation was mostly ascribed to the divergence between populations in Hokkaido and the Pacific coast in Honshu.

INTRODUCTION

Chum salmon (*Oncorhynchus keta*) is the most widely distributed species of salmon in the North Pacific Ocean (Salo, 1991). Spawning adults, like other anadromous Pacific salmon species, return to the natal river. Such restricted homing behavior will lead geographically distinct populations to partial genetic isolation.

Stock identification of salmon has been attempted by using tagging, scale characteristics, parasite tagging, thermal otolith marking, and/or protein genetic (allozyme) characters (Ishida *et al.*, 1989; Winans *et al.*, 1994; Willmot *et al.*, 1998; Urawa *et al*, 1998). Although variability of allozymes is beneficial for genetic stock identification (GSI) of chum salmon, allozyme analysis requires careful collection and handling of tissues (Park *et al.*, 1993). Furthermore, resolution of allozymes remains mostly at the regional- to continental-levels (Wilmot *et al.*, 1998).

Recently developed molecular techniques are a powerful replacement which compensates deficits of the use of allozymes with an increase in accuracy and resolution. Because mitochondrial (mt) DNA has higher sequence variability than single copy nuclear genes (Brown *et al.*, 1979) and clonal haploid inheritance, analysis of mtDNA has become a method of choice in phylogenetic and population genetic studies (Moritz *et al.*, 1987). In fact, mtDNA studies, mostly based on the analysis of restriction fragment length polymorphisms (RFLPs), has been conducted so far in many fish species including salmon (Meyer, 1993). Low levels of mtDNA sequence variation shown by RFLP analysis were reported in chum salmon (Park *et al.*, 1993) and other species of *Oncorhynchus* (Wilson *et al.*, 1987), providing the estimates of genetic divergence similar to those obtained from allozyme analyses (Seeb and Crane, 1999a).

The control region is considered to be the most variable portion of mtDNA, showing two to five times higher rates of nucleotide substitution than the protein-coding regions (Moritz *et al.*, 1987; Meyer, 1993). However, a low variation in the sequence of the mtDNA control region of chum salmon was reported after RFLP (Cronin *et al.*, 1993) and sequence analyses (Park *et al.*, 1993), probably because the samples in these studies were small or collected from small populations of restricted area.

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In the present study, we collected more than 500 chum salmon specimens from 11 rivers in the northern part of Japan to examine the variation of nucleotide sequence of mtDNA control region. Then, genetic variability within or among populations was estimated based on the sequence variation.

MATERIALS AND METHODS

Samples

Liver or blood samples were collected from 537 individuals of 12 populations which returned to their natal river in the latter half of breeding season from 1996 to 1999 (Table 1). Allozymes or other characters of chum salmons homed in these rivers were examined for more than a decade to monitor a possible annual difference in the stock (S. Urawa, personal communication). The homing migration of northern population is earlier than that of southern populations, and the peak runs in Hokkaido occur about one month earlier than those in Honshu in Japan (Salo, 1991). Early run may include descendants of populations which were transferred from rivers of other areas, particularly, in Honshu. Therefore, the samples from fish returned in the latter half of breeding season were used in the present study. Mostly late run fish were obtained from six rivers in Hokkaido. A possible genetic difference, if any, between early and late run fish was examined for those collected in the Tokoro River. Late run fish were obtained from five rivers in Honshu, i.e. two in Iwate and one in Miyagi Prefecture in the Pacific Ocean coast, and one each in Akita and Yamagata Prefectures in the Japan Sea coast (Table 1). The samples from the Otsuchi River were collected at random from juveniles of late run fish in the hatchery, which were obtained by the artificial fertilization of gametes from arbitrarily selected multiple parents and kept in large aquarium. The collected samples were stored at –80°C until DNA extraction.

DNA extraction

DNA was isolated from the stored specimens following the routine phenol-chloroform method (Sambrook *et al.*, 1989). About 50 µl of whole blood or liver homogenate was added to 500 µl sodium tris EDTA buffer (0.1 M NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH8.0) containing 500 µg/ml proteinase K and 0.5% SDS, and incubated at 37°C 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 24:1 chloroform:isoamylalcohol alone. DNA in aqueous phase was recovered by ethanol precipitation, dried in air, and dissolved in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5).

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

	Sampling location	Date of collection		N*
Hokkaido island				
A	Chitose River	14	Oct.1996	51
B	Tokushibetsu River	23	Sep.1997	51
C1	Tokoro River	20	Nov.1998	44
C2	Tokoro River	13	Oct.1999	49
D	Nishibetsu River	25	Sep.1997	41
E	Tokachi River	17	Oct.1996	46
F	Yurappu River	17	Nov.1998	40
Honshu island				
G	Tsugaruishi River, Iwate Pref.	10	Dec.1997	44
H	Otsuchi River, Iwate Pref.	8	Apr.1999	49
I	Koizumi River, Miyagi Pref.	21	Nov.1996	47
J	Kawabukuro River, Akita Pref.	18	Nov.1997	30
K	Gakko River, Yamagata Pref.	10	Dec.1996	45

* N is the number of samples.

PCR amplification

The control region of mtDNA was amplified by PCR in a 100 µl of reaction mixture containing 25–100 ng of template DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 1.5 mM $MgCl_2$, 250 nM each dNTP 250 nM of forward and reverse primers, 0.001% gelatin, and 1.25 U of *Taq* DNA polymerase (TaKaRa, Tokyo). PCR primers were designed based on the reported sequences of rainbow trout *O. mykiss* mtDNA (Zardoya *et al.*, 1995) and chum salmon control region (Shedlock *et al.*, 1992). They included forward primers, tRNAglu, 5'-AAC CAC CGT TGT TAT TCA ACT A-3'; tRNAthr-2, 5'-TCT TGT AAT CCG GAA GTC GGA-3'; and reverse primers, tRNAphe, 5'-CA(G/T) CTT CAG TG(T/C) TAT GCT TT-3'; tRNAphe-2, 5'-AAC AGC TTC AGT GTT ATG CT-3'. The tRNAglu is located at 5' to cytochrome *b* gene, but the others are in tRNA site flanking the control region. The condition of PCR amplification using a GeneAmp PCR System 9700 was as follows: preheating at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 58°C for 45 sec, and elongation at 72°C for 1 min, and completed with final extensions at 72°C for 7 min. The PCR products were purified by the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) after examining the size and quality with 1.5% agarose-gel electrophoresis and ethidium bromide staining.

Nucleotide sequence analysis

The mtDNA fragment amplified with tRNAglu and tRNAphe was cloned into vector pCR2.1-TOPO with the TOPO TA cloning™ system (Invitrogen, Carlsbad, CA). After cloning, the insert was analyzed for the sequence using a Hitachi SQ-5500L DNA sequencer (Hitachi, Tokyo). The nested fragment amplified with tRNAthr-2 and tRNAphe-2 was used as a template for direct sequence analysis. Sequence reaction was preformed using a Thermo Sequenase™ pre-mixed cycle sequencing kit (Vistra Systems, Sunnyvale, CA) according to the manufacturer's instruction. The forward and reverse sequence primers labeled with Texas Red included M13 reverse and T7 primers for cloned products and four primers for direct sequence analyses, which were designed based on own sequence data of the mtDNA control region. They included forward primer, tRNAthr-3, 5'-GGT TAA AAC CCT CCC TAG TG-3'; Okdl-L1, 5'-AGC TTG CAT ATA TAC AAG TGC A-3', and reverse primers, Okdl-H1, 5'-ATG GGT TCT CTG GAA TTC AA-3'; Okdl-H2, 5'-TGG GTA ACG AGC AAT AAG AT-3'. The 5' and 3' halves of the control region were analyzed using a pair of tRNAthr-3 and Okdl-H1 (or Okdl-H2) and a pair of Okdl-L1 and tRNAphe-2, respectively.

Data analysis

The sequence data were aligned by GENETIX-WIN version 4.0.1 (Software Development Co., Ltd, Japan) to find out nucleotide variations among the sequences of control region, from which the haplotypes of control region were defined. Phylogenetic relationship among the haplotypes was estimated by the neighbor-joining method (Saitou and Nei, 1987), based on genetic distance estimated by Kimura's two-parameter method (Kimura, 1980). A parsimony network which connects the observed haplotypes was drawn after Bandelt (1994).

The heterogeneity of the haplotype frequencies within and between geographic regions was evaluated using the contingency χ^2 test (Roff and Bentzen, 1989), with 17,000 Monte Carlo simulations generated by the CHIRXC program (Zaykin and Pudovkin, 1993). Some intra- and interpopulation parameters were calculated using Arlequin version 1.1 program package for the mtDNA sequence data (Schneider *et al.*, 1997). Haplotype diversity, being equivalent to expected heterozygosity for diploid data, was calculated for each population according to Nei (1973). 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; Excoffier *et*

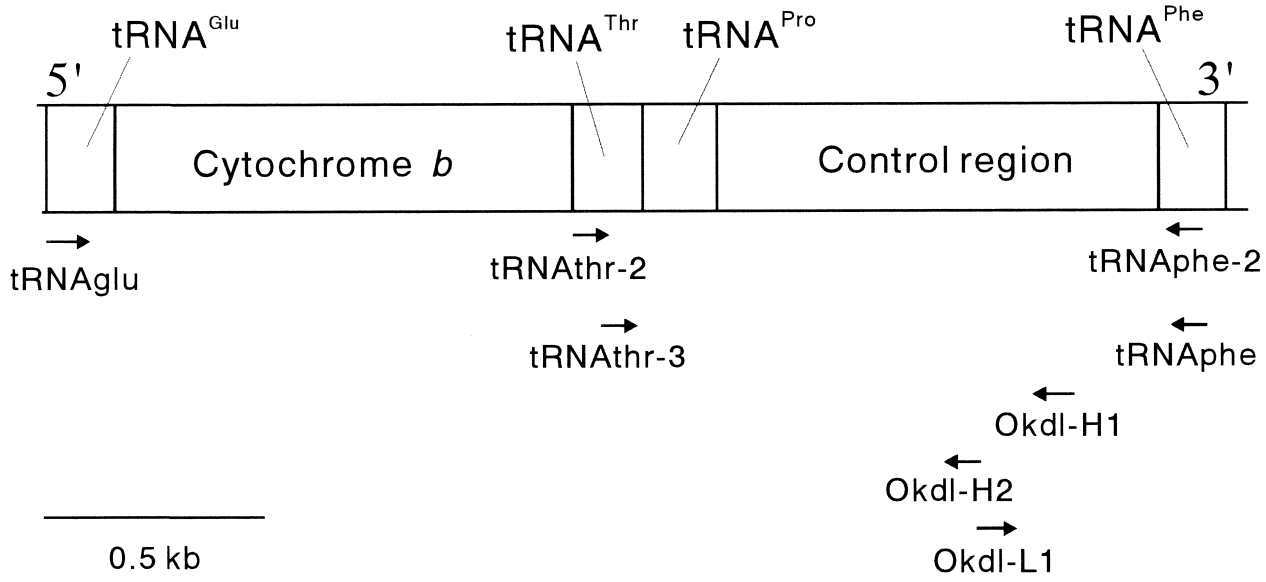

Fig. 1. Schematic diagram of mtDNA control region gene of *O. keta*. Horizontal arrows (tRNAglu, tRNAthr-2, tRNAthr-3, tRNAphe, tRNAphe-2, Okdl-L1, Okdl-H1, and Okdl-H2) show the positions of primer used for PCR amplification and sequence analysis.

al., 1992). For AMOVA, populations were grouped geographically by the above neighbor-joining algorithm, in which the obtained-topology was tested by a bootstrap analysis with 10,000 pseduoreplicate trees. Significance of the variance components and F_{ST} values was tested with a permutation method.

Pairwise F_{ST} values were calculated to estimate the genetic distance between populations, according to Slatkin (1995). The amount of gene flow (Nm) between populations was estimated using the approximation F_{ST} = 1/(2M+1), where M = Nm for haploid data. The estimation of Nm, however, was not conducted if its validity was not supported by the neutrality test after Tajima (1989) and by the regional equilibrium test after Hutchison and Templeton (1999).

RESULTS

Nucleotide sequences of the mtDNA control regions and flanking regions

About 2300 bp fragment was successfully amplified with a primer pair of tRNAglu and tRNAphe from DNA samples of two individuals. The PCR product was cloned and analyzed for the sequence by fluorochrome-labeled M13 reverse, tRNApro-3, T7, and Okdl-H1 primers. The base homology analysis with reported rainbow trout mtDNA sequence (Zardoya *et al*., 1995; EMBL/GenBank accession number L29771) revealed that the insert contains a 1002 bp control region sequence. It was flanked by a 3' part of cytochrome *b*, $tRNA^{Thr}$, and $tRNA^{Pro}$ at the 5' side and with a 5' part of $tRNA^{Phe}$ at the 3' side (DDBJ/EMBL/GenBank accession number AB039956), as shown in Fig. 1. This gene order around control region is in keeping with the previous findings in salmon (Shedlock *et al*., 1992; Zardoya *et al*., 1995). The three chum salmon tRNAs could be folded into a cloverleaf secondary structure which was assured with the tRNAscan-SE program (Lowe and Eddy, 1997), verifying that the obtained sequence is truly from mtDNA. The observed sequence of the control region was identical with the previously reported chum salmon sequence (Shedlock *et al*., 1992), except for a few substitutions and indels probably reflecting an interindividual variation.

Nucleotide sequence variation in chum salmon control region

About 1200 bp fragment was amplified with a primer pair of tRNAthr-2 and tRNAphe-2 on DNA samples from 30 individuals from the Chitose and the Otsuchi populations (Fig. 1). The direct sequence analysis on the fragment containing the entire control region and flanking tRNA sites was performed by fluorochrome-labeled tRNAthr-3, Okdl-L1, Okdl-H1, Okdl-H2, and tRNAphe-2. Multiple alignment of the obtained sequences revealed a cluster of variable sites (six of seven sites) within 500 bp from 5'-end of the chum salmon control region. Estimation of the 481 bp sequence in the 5' variable portion

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

Haplotype	30	57	96	108	154	194	231	386	395	471
1	T	A	—	A	C	A	T	G	C	A
2	·	·	·	·	·	T	·	·	·	·
3	·	·	·	·	·	·	C	·	·	·
4	·	·	·	·	·	·	·	·	·	C
5	·	·	A	·	·	·	·	·	·	·
6	·	·	·	·	·	·	·	—	A	·
7	·	·	·	·	G	·	·	—	A	·
8	·	·	·	·	·	·	C	—	A	·
9	C	·	·	·	·	·	·	·	·	·
10	C	T	·	·	·	·	·	·	·	·
11	C	·	·	T	·	·	·	·	·	·
12	C	·	·	·	·	·	C	·	·	·

The nucleotide at each position is given for haplotype-1 (OKDL-1). The hyphen represents the deletion and dots represents the same nucleotide as in the OKDL-1.

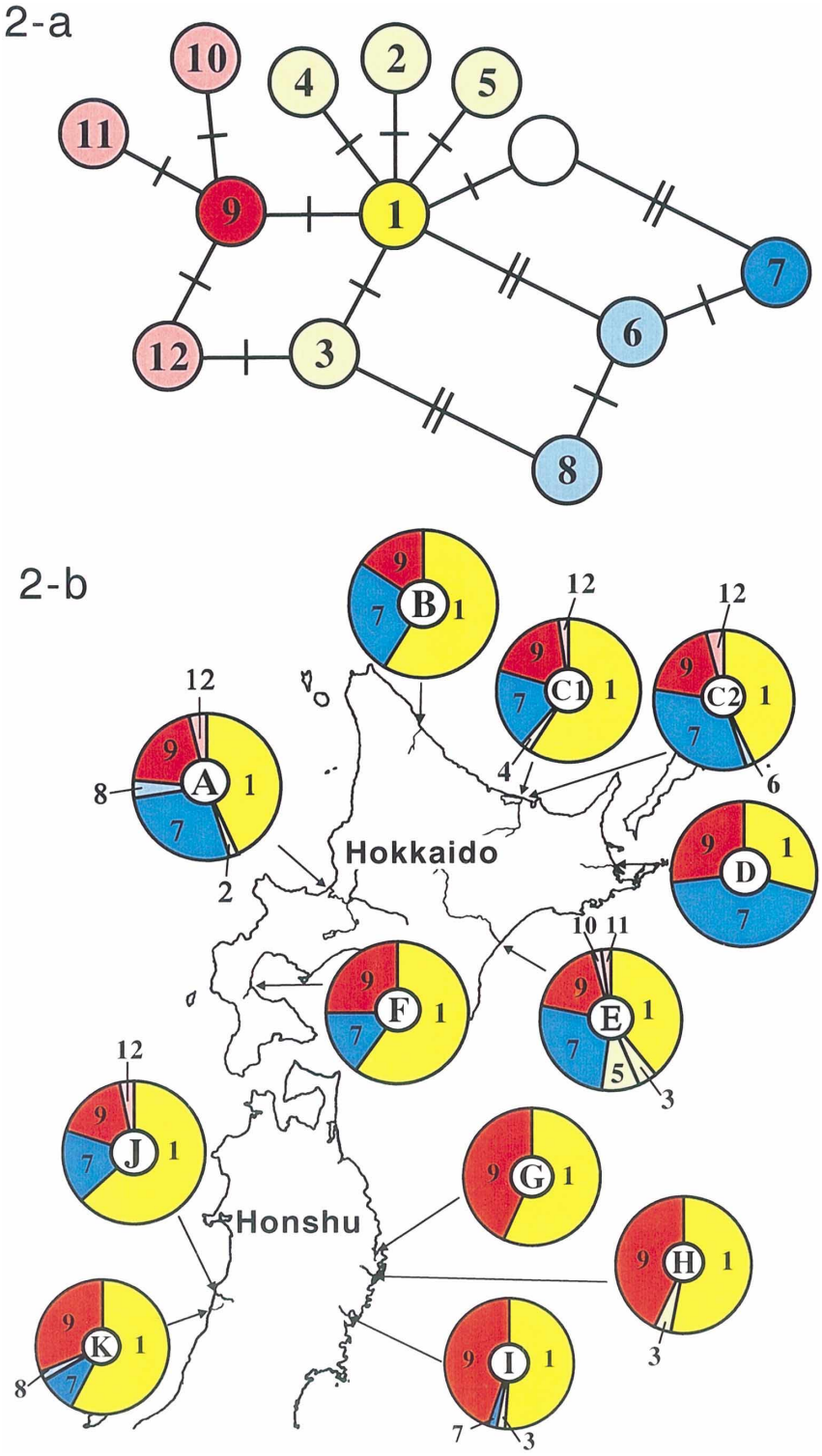

Fig. 2-a. Parsimony network of the mtDNA control region haplotypes (481 bp sequence) of *O. keta* presented in Table 2. Open circle without number is an intermediate between haplotypes OKDL-1 and -7, but was not found in 537 samples. Base substitutions and indels are indicated by slashes on the network.

Fig. 2-b. Geographical position of sampling site and distribution of mtDNA haplotypes. Haplotypes are shaded according to phylogenetic groups (see Fig. 2-a); OKDL-1 a yellow pie slice, OKDL-2 to -5 a light yellow pie slice, OKDL-7 a blue pie slice, OKDL-6 and -8 a light blue pie slice, OKDL-9 a red pie slice, and OKDL-10 to -12 a light red pie slice. See Table 1 for the name of rivers (or populations) denoted in capital.

disclosed 10 variable sites in a total of 537 individuals from 12 populations, defining 12 haplotypes designated as OKDL-1 to OKDL-12, as shown in Table 2. The observed variation included base substitutions and indels with an excess of transversion substitutions (six of eight substitutions). The sequences of entire chum salmon control regions were registered in the DDBJ/EMBL/GenBank with accession numbers AB039890 to AB039901. Nucleotide variation in the 3' downstream to the variable portion occurred only in the OKDL-4 haplotype (data not shown).

A parsimony network which connects the 12 control region haplotypes is presented in Fig. 2-a. The observed haplotypes of chum salmon could be grouped into three clades based on the nucleotide variation shown in Table 2, i.e., OKDL-1 to -5 in clade 1, OKDL-9 to -12 in clade 2, and OKDL-6 to -8 in clade 3. The T to C transition at nucleotide 30 separated the clade 2 from the clade 1, and a deletion at nucleotide 386 and the C to A substitution at nucleotide 395 discriminates clade 3 from clades 1 and 2, respectively. The T to C transition at nucleotide 231 in OKDL-3, -8, and -12 was homoplasmic. The neighbor-joining consensus tree, which was resulted from the bootstrap analysis of the 481 bp sequence and rooted with the corresponding region of the rainbow trout mtDNA, showed haplotype clustering that is identical to that observed in the network, but with less than 50% of bootstrap support for the nodes (data not shown).

Genetic differentiation among chum salmon populations

The distribution of 12 haplotypes among 12 populations of chum salmon is presented in Table 3 and Fig. 2-b. All the 12 haplotypes occurred in the seven Hokkaido populations, whereas only six haplotypes were found in the five Honshu populations. All the phylogenetic clades were found in the Kawabukuro, Gakko, and Koizumi populations, whereas the clade 3 was not found in the Tsugaruishi and Otsuchi populations (Fig. 2-b). The OKDL-1 and -9 were common in the 12 populations. The OKDL-7 haplotype was seen in all Hokkaido and three Honshu populations. Early and late run populations from the Tokoro river in Hokkaido showed essentially similar haplotype distribution, but differed in haplotype frequencies. The Hokkaido populations showed a higher level of haplotype diversity than the Honshu populations (Table 3).

Highly significant heterogeneity in the haplotype frequencies was revealed for the entire set of populations ($p<0.001$), seven Hokkaido populations ($p<0.001$), and Honshu populations ($p<0.005$) by contingency χ^2 test. Such significant heterogeneity was also observed for the set of populations from Hokkaido and Pacific coast or Japan Sea coast in Honshu ($p<0.001$), although no significant heterogeneity was shown for the populations within the two regions of Honshu ($p>0.3$). These findings suggest a greater genetic difference among populations between regions than within regions.

The extent of genetic differentiation at the different levels of geographic hierarchy was examined by the AMOVA. Using the neighbor-joining method, three population clusters, Pacific coast in Honshu, all Hokkaido and the Kawabukuro, and the Gakko, were obtained on the consensus tree, with more than 90% of bootstrap support for the clusters of Pacific coast in Honshu and the Gakko, respectively (Fig. 3). Early and late runs from the Tokoro River were separated from each other, but with less than 50% of the nodal value, and the heterogeneity in their haplotype frequencies was not significant by the χ^2 test ($p>0.5$). Taking together these observations and the results of the above contingency χ^2 test, grouping of three geographic regions, i.e. Pacific coast in Honshu, Hokkaido, and Japan Sea coast (including both the Kawabukuro and Gakko) was considered to be most appropriate for AMOVA. The AMOVA analysis indicated that the magnitude of variance among three regional groups was moderate but significant (11.32%, $P<0.001$). However, the variance among populations within regions was very low (<1%, $P>0.1$). This fact suggests that the populations within regions are geneti-

Table 3. Distribution of mtDNA control region haplotypes and haplotype diversity within the 12 populations calculated from mtDNA haplotype frequencies

	Number of individuals with haplotype												
	1	2	3	4	5	6	7	8	9	10	11	12	Haplotype diversity
Hokkaido island													
Chitose	22	1	0	0	0	0	14	2	10	0	0	2	0.71±0.04
Tokushibetsu	30	0	0	0	0	0	13	0	8	0	0	0	0.58±0.05
Tokoro	26	0	0	1	0	0	8	0	8	0	0	1	0.60±0.07
Tokoro-early	21	0	0	0	0	1	16	0	9	0	0	2	0.69±0.04
Nishibetsu	12	0	0	0	0	0	18	0	11	0	0	0	0.67±0.03
Tokachi	18	0	2	0	4	0	12	0	8	1	1	0	0.75±0.04
Yurappu	24	0	0	0	0	0	6	0	10	0	0	0	0.57±0.06
Honshu island													
Tsugaruishi	25	0	0	0	0	0	0	0	19	0	0	0	0.50±0.03
Otsuchi	26	0	2	0	0	0	0	0	21	0	0	0	0.54±0.03
Koizumi	24	0	1	0	0	0	1	0	21	0	0	0	0.55±0.03
Kawabukuro	19	0	0	0	0	0	5	0	5	0	0	1	0.56±0.09
Gakko	26	0	0	0	0	0	4	1	14	0	0	0	0.57±0.05
Total	273	1	5	1	4	1	97	3	144	1	1	6	0.64±0.01

cally indistinguishable. Most of the variation occurred within populations (87.70%, P<0.0001). The results of AMOVA imply that the three regional groups of populations are genetically differentiated from each other.

The increased level of genetic isolation among populations between regions was suggested for Hokkaido, except for the Yurappu, and Pacific coast in Honshu by high F_{ST} values (0.103 to 0.328) compared with other pairwise estimate, as shown in Table 4. These results imply a low level of gene flow among populations between Hokkaido and Pacific coast in Honshu. Tajima's test (1989) supported the neutrality for the mtDNA variations in all populations (p<0.01, D= –2.092 to 2.840 or p<0.05, D= –1.734 to 2.130), except for the one in the Nishibetsu population (p>0.1, D= 3.7568). In addition, the relationship between F_{ST} and geographic distance in the seven Hokkaido populations inferred a lack of regional equilibrium,

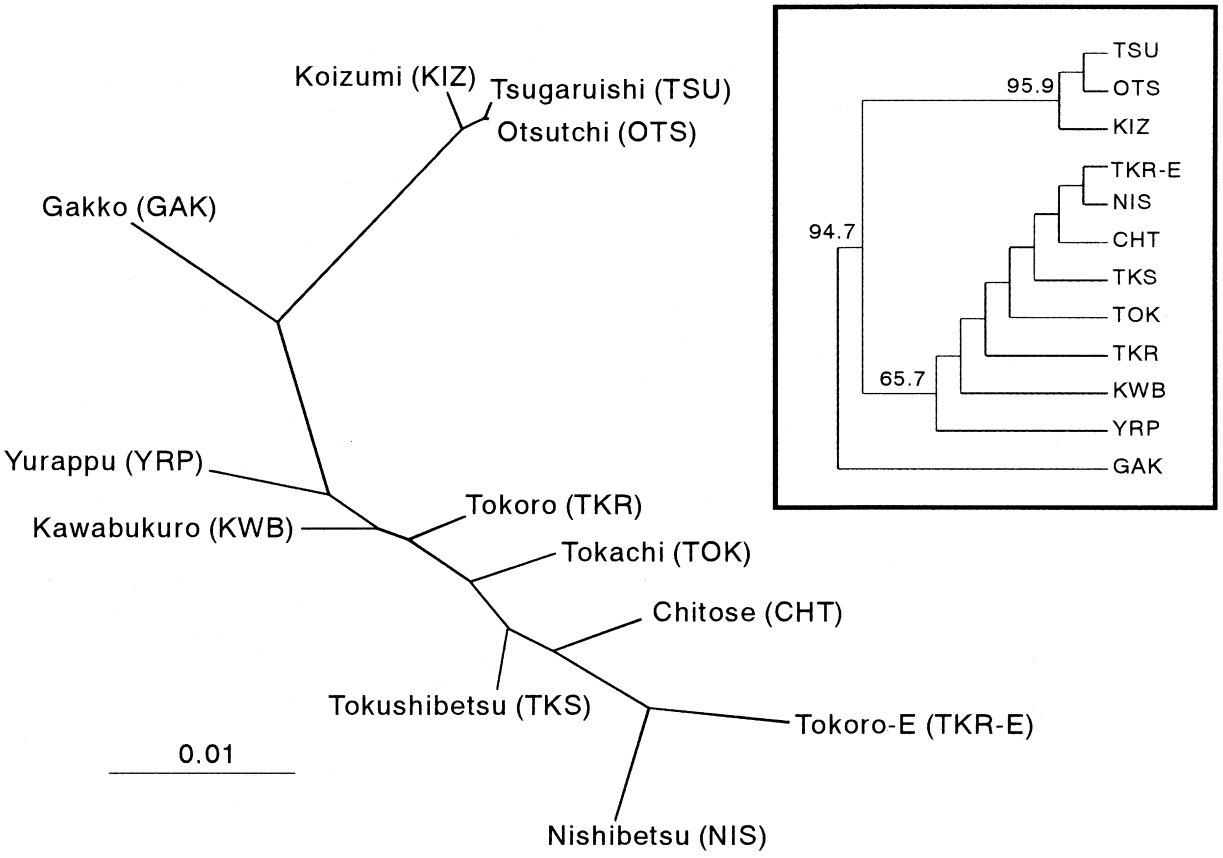

Fig. 3. Neighbor-joining tree and phenogram (inset) of chum salmon populations based on the haplotype frequencies and average nucleotide diversities between populations (Saitou and Nei, 1987). Nodal numbers in the phenogram are bootstrap values based on 10000 replications.

Table 4. Pairwise estimates of F_{st} based on mtDNA sequence data

			Hokkaido Island					Honshu Island			
Population	CHT	TKS	TKR	TKR-E	NIS	TOK	YRP	TSU	OTS	KIZ	KWB
Hokkaido Island											
Chitose											
Tokushibetsu	–0.007										
Tokoro	0.009	–0.007									
Tokoro-early	–0.017	–0.003	0.026								
Nishibetsu	0.013	0.045	0.093	–0.005							
Tokachi	–0.012	–0.012	–0.005	–0.006	0.033						
Yurappu	0.026	0.009	–0.019	0.048	0.121	0.008					
Honshu Island											
Tsugaruishi	0.193	0.199	0.125	0.235	0.328	0.163	0.090				
Otsuchi	0.190	0.198	0.122	0.234	0.328	0.162	0.088	–0.019			
Koizumi	0.167	0.175	0.103	0.208	0.296	0.141	0.070	–0.018	–0.018		
Kawabukuro	0.009	–0.007	–0.028	0.029	0.099	–0.006	–0.026	0.125	0.119	0.100	
Gakko	0.061	0.051	0.003	0.091	0.173	0.039	–0.014	0.037	0.035	0.023	–0.006

i.e. absence of isolation by distance (data not shown). From these observations, estimation of Nm was not conducted in the present study. Correlation between genetic and geographic distance was not tested for other regions, because the number of populations was small and pairwise F_{ST} values between populations were mostly not significant.

DISCUSSION

The present mtDNA analysis demonstrated: 1) 10 variable positions in the nucleotide sequences of about 500 bp from the 5' end of control region (Table 2), defining a total of 12 haplotypes among more than 500 individuals from 12 Japanese populations of chum salmon (Table 3); 2) probable genetic differentiation among three regional groups of Japanese populations of chum salmon, i.e. Hokkaido, the Pacific coast in Honshu, and Japan Sea coast in Honshu; 3) greater genetic variation in the populations of Hokkaido than those of Honshu; and 4) moderate but significant genetic isolation of the populations between Hokkaido and the Pacific coast in Honshu.

The sequence variation detected in the control region of Japanese chum salmon was similar to or even higher than the diversity in other *Oncorhynchus* species including *O. mykiss, O. kisutch* and *O. tshawytscha* (Nielsen *et al.*, 1994). Thus, the number of observed haplotypes is apparently greater than those obtained from the previous RFLP analyses on chum salmon mtDNA (Cronin *et al.*, 1993; Park *et al.*, 1993; Bickham *et al.*, 1995; Seeb and Crane, 1999b). These findings indicate an increased potential of mtDNA sequence analysis to estimate the genetic structure of chum salmon populations.

The 12 haplotypes were phylogenetically classified into three clades (Fig. 2-a). Because of their prevalence in the examined populations, the haplotype OKDL-1, -9 and -7 are considered to be ancestral one within the clade 1, 2 and 3, respectively, and hence parental to the other haplotypes less frequent in the each clade. However, the frequency of OKDL-7 was low in Honshu populations and this haplotype was not detected in the Tsugaruishi and Otsuchi (Fig. 2-b). The decreased number of haplotypes in these two populations suggest that they might have gone through the decrease of population size, that resulted in extinction of the lineage with the OKDL-7 and other haplotypes.

Results of AMOVA were corroborated by the contingency χ^2 test for the heterogeneity in the haplotype frequencies among populations. Genetic differentiation among the three regional groups of Japanese chum salmon, as inferred from the AMOVA, was also suggested by the previous allozyme analysis (Kijima and Fujio, 1979). Little genetic differentiation among populations within regions may partly be associated with a level of straying rate (about 14%) in spawning migration of chum salmon (Salo, 1991), which could cause a gene exchange between rivers. The estimates of haplotype diversity and pairwise population F_{ST} strongly suggest that the regional differentiation is mostly ascribed to the divergence between populations in Hokkaido and the Pacific coast in Honshu. Probable low gene flow between Hokkaido and the Pacific coast in Honshu may be contributed by the differences in the route of spawning migration (Kijima and Fujio, 1982; Okazaki, 1986) and run timing (Salo, 1991). In fact, the previous allozyme study suggested that separation by at least 600 km is required for genetic differentiation to occur (Kijima and Fujio, 1982). This estimation well fits the minimum distance between the populations of Hokkaido, expect for the Yurappu, and the Pacific coast in Honshu.

The estimated genetic structure of Japanese chum salmon populations may reflect either the effect of historical factors such as the glacial advancement and resulting shift of the species range to southward or contemporary forces such as human-mediated extensive transplantation of stocks, or both. In fact, the Hokkaido and Honshu populations have undergone extensive hatchery operations and transplantation of eggs and fry from one river population to another in history of raising commercial salmon production (Kijima and Fujio, 1982). Such an extensive transplantation of stocks from Hokkaido might be a cause of the Kawabukuro being positioned within a cluster of Hokkaido populations (Fig. 3). Absence of isolation by geographic distance in Hokkaido populations implies a possible contribution of an artificial transplantation of stocks among rivers. The lack of such regional equilibrium also suggests little gene flow and divergence mostly by drift in the formation of Hokkaido populations (Hutchison and Templeton, 1999). The regional equilibrium test using a larger number of populations in Honshu should be performed to examine the factor(s) involved in the formation of their genetic structure. In any case, mtDNA analysis on foreign populations such as Russia, USA, and Canada would be helpful to understand how population structures in Japanese chum salmon were established.

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