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Genetic variations and differences among the chum salmons from Korea and several countries determined by mitochondrial DNA analysis

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Abstract

In order to estimate the genetic variations and differences among the salmons from Korea, Canada, Russia and Bering Sea, we analyzed the haplotype (H) and nucleotide diversities (π) in the mitochondrial control regions. 187 samples were collected from seven populations (two populations from Korea, one from Bering Sea and Russia, respectively and three from Canada). The polymerase chain reaction was used to amplify 549bp segments (hypervariable region) of the mitochondrial control region. PCR/direct sequencing data indicated 28 distinct haplotypes in the salmons from Korea, Canada, Russia and North-Pacific Bering Sea. While the haplotype 2 was the main type for two populations from Korea, the haplotype 3 was the main for 4 populations from Canada (F and K), Russia and North-Pacific Bering Sea and haplotype 21 for another population from Canada (N). In view of the results so far achieved, the rate of upstream migrating salmons to Korean waters increased every year and there might be chances for Korean populations to enter and mix with the North-Pacific Bering Sea populations.

Introduction

Salmons have been used as important food resources of several well-developed fisheries countries such as the U.S., Japan, Russia, Canada and Norway where diverse studies on productivities and efficiency of salmon releasing have been performed for a long time. Stocking streams with fries of salmons have launched in Korea since 1970s to build up coastal fisheries resources and drawn more and more concerns about values and importance of salmons as food resources.

In Korean waters, the salmonid fish has four species of the Oncorhynchus genus : cherry salmon (*O. masou masou*), Ishikawa's cherry salmon (*O. masou ishikawai*), chum salmon (*O. keta*) and rainbow trout (*O. mykiss*) are fertilized and nursed artificially to stock streams with their fries.

While studies on these higher value added aquaculture species have been mainly focused on taxonomy with morphological characteristics or cell-leveled genetics and biochemistry to create polyploidy or apply to aquaculture (Hong *et al*., 1994; Myoung and Kim, 1996; Park *et al*., 1997), population genetics to identify species or population structures have been rarely studied. But population genetics with new molecular biological methods must be supported to utilize and manage these ocean-wide migrating species among neighboring countries.

Methods to distinguish an individual, population and species from another ones form the basis of many studies in population biology, genetics and ecology. With the advent of new molecular biological techniques, there has been increasing emphasis on the use of DNA characteristics as genetic markers.

Recently, the analyses of mitochondrial or microsatellite DNA became very useful tools to justify the genetic differences among species and populations (Thomas and Beckenbach, 1989; Beckenbach, 1991; McVeigh and Davison, 1991; Kitano *et al*., 1997). Mitochondrial DNAs have double helix spiral structures, large number of copies in the cells, small enough for separations and analyses and 5 to 10 times faster than nucleic DNAs in the evolution. All of these characteristics give advantages for population genetics (Brown *et al*. 1982). Most of gene sequences consisting of mitochondrial DNAs are very conservative and especially ribosomal genes and protein coding genes existing in the mitochondrial DNAs of all organisms have both conservative and variable features in gene sequences so that they can be applied to analyses for variations in populations or similarities among species (Bartlett and Davidson, 1991; Beckenbach, 1991; Carr and Marshall, 1991).

In this report, we have analyzed the partial control region sequences of salmon samples. Based on these sequence data, we provide information on the level of mitochondrial DNA sequence variation and differences among salmons from Korea, Canada, Russia and North-Pacific Bering Sea for the uses in genetic stock identification and phylogenetic reconstructions.

Materials and Methods

Fish samples

The chum salmon, *Oncorhynchus keta*, samples were collected by Yangyang Inland Fisheries Institute, Korea in 2004 and 2005. Canada and Russia presented their samples to this study as the NPAFC's regulation on mutual exchanges of samples and the North-Pacific Bering Sea samples were obtained from salmon BASIS plan in 2004 (Table 1). Tissues were removed from freshly sacrificed fish in the field, put immediately on dry ice and then kept at -80□ until DNA extractions.

PCR amplification

Mitochondrial control region was amplified by the PCR using standard protocols (McVeigh and Davison, 1991). PCR amplification was performed with 0.2 - 0.5g of template DNA in a reaction mixture of 50l 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: the denaturation step was at 94□for a minute, the annealing step was at 55□ for a minute and the polymerization reaction was performed at 72□ for 2 minutes. Oligonucleotide primer pairs were used Pro-L (5'-CTACCTCCAACTCCCAAAGC-3') and Chum-3 (5'-ACTTTCTAGGGTCCGTCTTA-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 seconds; 50□ for 5 seconds; 55□ for 4 minutes 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.

Sequence data analysis

The sequences were aligned, compared and translated using the program DNASIS Version 2.5 (Hitachi Software Engineering Co., Ltd.). Nucleotide sequences of all salmon samples were used in phylogenetic analysis. The bootstrap analysis (1000 replicates) was performed with the taxon input order randomized once for each replicate. UPGMA bootstrap trees (Saitou and Nei, 1987) were constructed from Kimura's two-parameter (Kimura, 1980) corrected distance matrices using the program NEIGHBOR in PHYLIP (Felsenstein, 1993).

Results and Discussions

The partial sequences of mitochondrial DNA control region from Korea, Canada, Russia and North-Pacific Bering Sea salmons were determined using PCR and direct sequencing. The analyzed size of the molecules was 549 nucleotides.

Numbers and frequencies of haplotype in 7 populations from 4 regions are shown in Table 2. While the haplotype 2 was the main type for two populations from Korea, the haplotype 3 was the main for 4 populations from Canada (F and K), Russia and North-Pacific Bering Sea and haplotype 21 for another population from Canada (N). Those results revealed that the salmons migrating to Korean waters are dissimilar to the populations migrating to the other regions. However N population of Canada might be identified as a wholly separated subspecies because of no common haplotype. Although Korean populations were genetically different from the other populations as described earlier, the haplotype 3 accounted for about 8% of the population 2004 and increased to 20% in the population 2005 which implied the possibility for Korean populations to be mixed with the other populations in the Bering Sea and it is expected to be clear in the population 2006 and following years.

Haplotype (H) and nucleotide (π) diversities of every each of populations are shown in Table 3. The haplotype diversities of two Korean populations were 0.295 (2004) and 0.433 (2005), those of three Canadian populations were ranged from 0 (K) to 0.423 (F) and Russian population was 0.495. The North Pacific Bering Sea population had the highest value (0.778) and it could be inferred the mixing among several genetically different populations induced such

a high value of haplotype diversity. Lower values of Korean populations mean higher rates of upstream migration every year in this population.

Genetic distances in inter- and intra-populations are described in Table 4. With regard to genetic distances in intra-populations, Korean populations had lower value (0.00116) than Russia (0.00151) or North-Pacific Bering Sea (0.00457) populations which mean that Korean populations have more fixed genes than the others. In inter-populations genetic distances, Russian populations were genetically close to North Pacific Bering Sea populations but the genetic distance between Korean and North Pacific Bering Sea populations was very far. This situation is thought to be due to the differences in main haplotypes and in breeding groups.

Figure 1 shows the relationship of genetic similarity among 7 populations in UPGMA (A) and N-J (B) phylogenetic tree, which presented the same trend in Table 4.

As shown in Table 5, there were significant differences in P values among the populations except the Korean population 2004 and North-Pacific Bering Sea population. These patterns were shown in F_{st} values also and presented the mixing possibility between Korean and North-Pacific Bering Sea populations.

In view of the results so far achieved, the rate of upstream migrating salmons to Korean waters increased every year and there might be chances for Korean populations to enter and mix with the North-Pacific Bering Sea populations. It can be elaborated when various analysis methods like microsatellite DNA and cooperative studies among the neighboring countries are followed.

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Table1. Sample list of chum salmon, Onchorhyncus keta, collected from Korea, Canada, Russia and North-Pacific

Area	Population (Abbr.)	Date	No. of samples
Korea	Namdaechun (2005)	2005/11	66
	Namdaechun (2004)	2004/10	13
Canada	Nuiukluk River (NUI)	2004	14
	Kwiniuk River (KWI)	2004	13
	Fish River (FIS)	2004	13
Russia	Tarani River (TAR)	2004	14
North-Pacific	Bering Sea (BER)	2004	54
Total No. of Samples		187	

Table 2. Comparisons of mtDNA haplotypes and frequencies among 7 populations from Korea, Canada, Russia and North-Pacific

Haplo-	Korea		Canada			Russia	N-P
type	2005	2004	NUI	FIS	KWI	TAR	BER
1	5(0.0758)	0	0	0	0	0	3(0.0556)
2	48(0.7273)	11(0.8462)	0	0	0	0	9(0.1667)
3	13(0.1970)	1(0.0769)	0	10(0.7692)	13(1.000)	10(0.7143)	24(0.4444)
4	0	1(0.0769)	0	0	0	0	0
5	0	0	0	0	0	0	1(0.0185)
6	0	0	0	0	0	0	1(0.0185)
7	0	0	0	0	0	0	1(0.0185)
8	0	0	0	0	0	0	1(0.0185)
9	0	0	0	0	0	0	1(0.0185)
10	0	0	0	0	0	0	1(0.0185)
11	0	0	0	0	0	0	1(0.0185)
12	0	0	0	0	0	0	1(0.0185)
13	0	0	0	0	0	0	1(0.0185)
14	0	0	0	0	0	0	1(0.0185)
15	0	0	0	0	0	0	1(0.0185)
16	0	0	0	0	0	2(0.1429)	3(0.0556)
17	0	0	0	0	0	0	1(0.0185)
18	0	0	0	0	0	0	1(0.0185)
19	0	0	0	0	0	0	1(0.0185)
20	0	0	0	0	0	0	1(0.0185)
21	0	0	11(0.7857)	0	0	0	0
22	0	0	1(0.0714)	0	0	0	0
23	0	0	1(0.0714)	0	0	0	0
24	0	0	1(0.0714)	0	0	0	0
25	0	0	0	1(0.0769)	0	0	0
26	0	0	0	1(0.0769)	0	0	0
27	0	0	0	1(0.0769)	0	1(0.0714)	0
28	0	0	0	0	0	1(0.0714)	0

□ The shaded cells indicate the main haplotypes in the populations and frequency values are in the parenthesis.

Table 3. Comparisons of haplotype (H) and nucleotide (π) diversities among 7 populations from Korea, Canada, Russia and North Pacific

	Korea		Canada			Russia	N-P
	2005	2004	NUI	FIS	KWI	TAR	BER
No. of sequences	66	13	14	13	13	14	54
No. of haplotypes	3	3	4	4	1	4	19
H	0.433	0.295	0.396	0.423	0.000	0.495	0.778
π	0.00142	0.00083	0.00129	0.00139	0.00000	0.00151	0.00454
Sum (H)							
Sum (π)	0.00225		0.00268			0.0015	0.00454

Table 4. Genetic distances among 7 populations from Korea, Canada, Russia and North-Pacific calculated by Kimura's two-parameter method

		Korea		Canada			Russia	N-P
		2005	2004	NUI	FIS	KWI	TAR	BER
Korea	2005	0.00142	0.00003	0.00190	0.00187	0.00233	0.00192	0.00086
	2004	0.00116	0.00084	0.00259	0.00255	0.00306	0.00261	0.00133
Canada	NUI	0.00326	0.00365	0.00129	-0.00008	0.00000	-0.00004	0.00312
	FIS	0.00327	0.00366	0.00126	0.00139	0.00000	-0.00004	0.00316
	KWI	0.00304	0.00348	0.00065	0.00070	0.00000	0.00002	0.00265
Russia	TAR	0.00339	0.00378	0.00137	0.00141	0.00078	0.00151	0.00320
N-P	BER	0.00385	0.00403	0.00019	0.00017	0.00037	0.00016	0.00457

※ Diagonal: Intra-populational distance d_{ii}

※ Lower left: Inter-populational distance d_{ij}

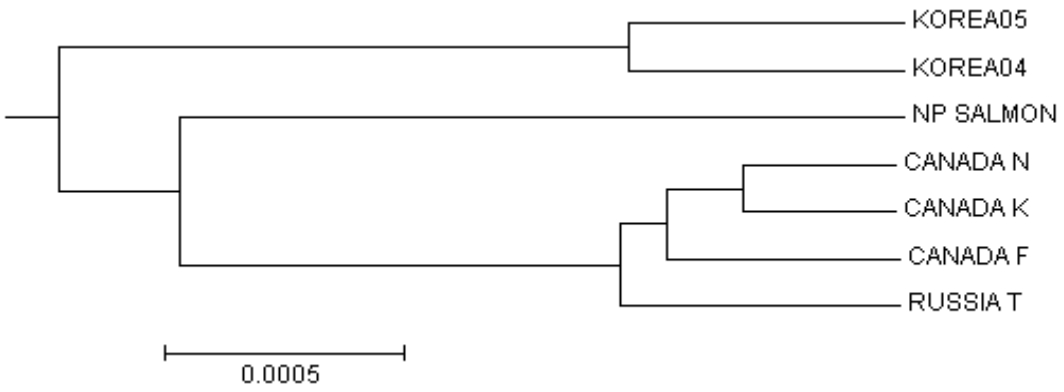
※ Upper right: Net inter-populational distance $d_{ij} - (d_{ii} + d_{jj})/2$

Table 5. Estimated *Fst* (below diagonal) and *P* (upper diagonal) values among 7 populations from Korea, Canada, Russia and North-Pacific

		Korea		Canada			Russia	N-P
		2005	2004	NUI	FIS	KWI	TAR	BER
Korea	2005	-	0.10680	0.00000*	0.00000*	0.00000*	0.00000*	0.00000*
	2004	0.02623	-	0.00000*	0.00020*	0.00000*	0.00000*	0.07110
Canada	NUI	0.58334	0.70845	-	0.00000*	0.00000*	0.00000*	0.06102
	FIS	0.76674	0.88000	0.00000	-	0.21670	0.73750	014043
	KWI	0.57021	0.69591	-0.06299	0.00000	-	0.09220	0.05588
Russia	TAR	0.94815	0.95148	0.95053	0.95827	0.94985	-	0.93153
N-P	BER	0.22264	033070	0.00000	0.39530	0.82020	0.65130	-

* Significant differences at 95% level of confidence

(A)



(B)

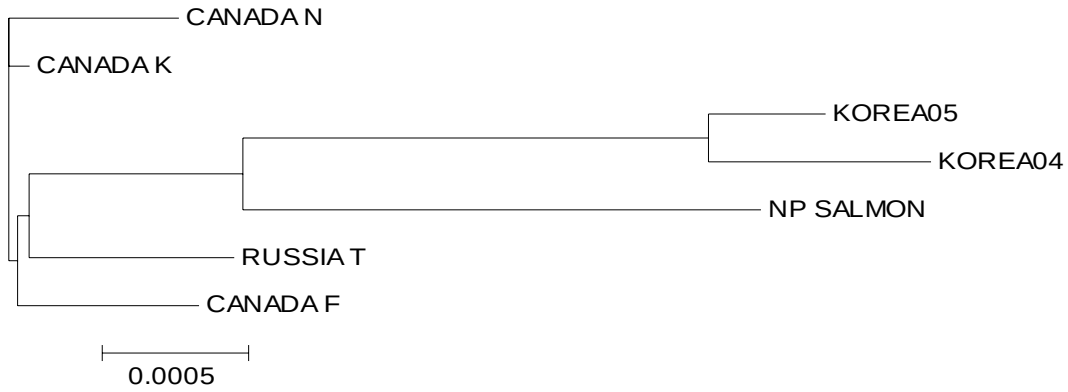


Fig. 1. UPGMA (A) and N-J (B) Phylogenetic tree of genetic distance among 7 populations calculated on the basis of the mitochondrial control region sequences.