

Preliminary mitochondrial DNA analysis between Korea and North-Pacific Bering sea salmon

by

Jung-Youn PARK, Ki-Back SEONG*, and Young-Hee HUR**

Biotechnology Research Center, National Fisheries Research & Development Institute, Busan 619-900,
Korea

*Aquaculture Division, South sea Fisheries Research Institute, Yosu, 556-823, Korea

**Salmon Research Team,, East sea Fisheries Research Institute, YangYang 626-900, Korea

Submitted to the

NORTH PACIFIC ANADROMOUS FISH COMMISSION

By the

Korea

October 2004

THIS PAPER MAY BE CITED IN THE FOLLOWING MANNER:

Davis, N.D., J.L. Armstrong., and K.W. Mayers. 2004. Bering Sea salmon diet overlap in Fall 2002 and potential for interactions among salmon. NPAFC Doc. 825. Biotechnology Research Center, NFRDI, Busan. 6 p.

Preliminary mitochondrial DNA analysis between Korea and North-Pacific Bering sea salmon

Abstract

In order to estimate the genetic differences between Korean and North-Pacific Bering sea salmon, we analyzed the haplotype diversity(H) and nucleotide diversity(π) in the mitochondrial control regions. 65 samples were collected one location of Korea and eight stations of North-Pacific Bering sea. The polymerase chain reaction was used to amplify a 550bp segment (hypervariable region) of the mitochondrial control region. PCR/direct sequencing data indicated the following results; there were 20 distinct haplotypes in Korea and North-Pacific Bering sea salmon. Of the 20 haplotypes, only three haplotypes are revealed in Korea salmon samples. The calculated average haplotypic diversity value are 0.34545 in Korea sample and 0.79315 in North-Pacific Bering sea samples. From these results, we could estimate the possibility of the existence of unique Korea salmon stock different from North-Pacific Bering sea salmon stocks.

Introduction

Methods for distinguishing between individuals, populations, and species form the basis of many investigations in population biology, genetics and ecology. With the advent of new molecular biological techniques, there has been an increasing emphasis on the use of DNA characteristics as genetic markers.

The salmon is treated as an important industrial resource in several advanced fisheries countries like as USA, Japan and Canada etc and also they are deeply involved in the researches from various points of view like as productivity increasing and release effectiveness etc. In our country, also, large-scale fry-releasing project has been executed from 1970th and as the results of the project we have been groping for the coastal fishery resources creation and proliferation, so that the concerning on value for foods and also the industrial importance is getting increased.

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*).

In this report, we have analysed the partial control region sequences of salmon samples. Based on these sequence data, we provide information on the level of mtDNA sequence variation between Korea and North-Pacific Bering sea salmon for use in genetic stock identification and phylogenetic reconstructions.

Materials and methods

Fish samples

Korea fish samples of chum salmon, *Oncorhynchus keta*, were obtained from Yangyang Inland Fisheries Institute, Kangwon-do, Korea. And the North-Pacific Bering sea samples were obtained from salmon BASIS plan in 2004. Tissues were removed from freshly sacrificed fish in the field, put immediately on dry ice and then kept at 80°C until DNA extraction.

PCR amplification

Mitochondrial control region was amplified by the PCR using a standard protocols (McVeigh et al., 1991). PCR amplification was performed with 0.2 - 0.5µg of template DNA in a reaction mixture of 50µl containing 1.25 units of Taq DNA polymerase (Ex Taq™, 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°C for 1 min, the annealing step was at 55°C for 1 min, and the polymerization reaction was performed at 72°C for 2 min. Oligonucleotide primer pairs were used CB3R-L (5'-CATATTAACCCGAATGATATTT-3') and 12SD-2RV (5'-TCGTRTGACCGCGGTGGCTG-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°C for 10s; 50°C for 5s; 55°C for 4min 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 DNASIS ver. 2.5 program (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 NEIGHBOR program in PHYLIP (Felsenstein, 1993).

Results

The partial sequences of mitochondrial DNA control region from Korea and North-Pacific Bering sea salmons were determined using PCR and direct sequencing. The analyzed size of the molecules was 550 nucleotides.

Table 1 shows the number of analyzed samples and the target area of salmons collected from Korea and North-Pacific Bering sea. Because the sample number tested was so small that the reliable results could not be attained. It will be proceeded with the higher number of samples in the future.

Table 2 shows the haplotypes of the samples from Korea and North-Pacific Bering sea. As you can see here, three haplotypes were obtained in Korea samples. Among them, haplotype 1 showed over 90% occupancy. Whereas, 19 haplotypes were obtained from the samples from North-Pacific Bering sea and the haplotype 3 except the sample from station 02 and 11 showed the major occupancy.

In the next, Table 3 shows the haplotype diversity (H) and nucleotide diversity (π) of the samples were calculated. As you can see here, the haplotype diversities of Korea and North-Pacific Bering sea samples were 0.54545 and 0.79315, respectively, showing that the haplotype diversity of Korean samples were lower

than that of North-Pacific Bering sea samples. However, the sample from station 25 of the N-P Bering sea showed lower haplotype diversity (0.00046). It is speculated that the station 25 might be the intermixing area of Korean and N-P Bering sea salmon or the first excursion point of Korean salmon. Based on these results, it is interpreted that Salmon (coming) Korea is the single group fixed on gene or have a small effective population size for mating.

Table 4 shows the genetic distances of the intrapopulation and interpopulation from Korean and North-Pacific Bering sea samples. This result also shows that the genetic distance of the intrapopulation from Korea is lower than that of N-P Bering sea.

To see the genetic differences between Korean and Bering-sea samples, X^2 examination was occurred in Table 5. Except the station 5, all of the samples between Korea and North-Pacific Bering sea showed the genetic differences.

Based on above results, we can conclude that the salmon coming to Korea is a genetically different group from that excursing around the North-Pacific Bering sea, also it will not be in charge of increasing the population of salmon in North-Pacific Bering sea.

Finally, we are going to notify one thing here that we may have different results when the sample number is increased. It is expected that the proceeded results will be extracted in next year.

References

- Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, 16, 111-120.
- McVeigh, H. P. and W. S. Davidson. 1991. A salmonid phylogeny inferred from mitochondrial cytochrome b gene sequences, *J. Fish Biol.*, 39(Suppl. A), 277-282.
- Felsenstein, J. 1993. *PHYLIP (Phylogeny Inference Package)*, version 3.5c. Department of Genetics, University of Washington, Seattle.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4, 406-425.

Table 1. Sample list of chun salmon, *Onchorhynchus keta*, collected from Korea and North-Pacific Bering sea

Sampling Area	Date	latitude	longitude	No. of samples
Korea namdaechun	200410XX			11
North-Pacific St 02	20040626	51°49' N	170°00' W	3
North-Pacific St 05	20040628	55°04' N	170°01' W	8
North-Pacific St 07	20040629	57°58' N	174°42' W	6
North-Pacific St 11	20040701	54°10' N	175°02' W	5
North-Pacific St 14	20040703	50°38' N	180°00'	7
North-Pacific St 17	20040704	53°22' N	179°49' W	9
North-Pacific St 21	20040706	57°20' N	179°53' W	8
North-Pacific St 25	20040708	52°58' N	175°16' E	8
Total No. of Samples				65

Table 2. Comparison of mtDNA haplotypes from three populations; past commercial samples and by-catch samples

Haplotypes	Sampling area								
	Korea	S02	S05	S07	S11	S14	S17	S21	S25
1	9	0	1	1	2	1	2	2	0
2	1	0	0	0	0	0	0	0	0
3	1	1	4	2	0	2	5	3	7
4	0	1	0	0	0	0	0	0	0
5	0	1	0	0	0	0	0	0	0
6	0	0	1	0	0	0	0	0	0
7	0	0	1	0	0	0	2	0	0
8	0	0	1	0	0	0	0	0	0
9	0	0	0	1	0	0	0	0	0
10	0	0	0	1	0	0	0	0	0
11	0	0	0	1	0	0	0	0	0
12	0	0	0	0	1	0	0	0	0
13	0	0	0	0	1	0	0	0	0
14	0	0	0	0	1	0	0	0	0
15	0	0	0	0	0	1	0	0	0
16	0	0	0	0	0	1	0	1	1
17	0	0	0	0	0	1	0	0	0
18	0	0	0	0	0	1	0	0	0
19	0	0	0	0	0	0	0	1	0
20	0	0	0	0	0	0	0	1	0
No. of samples	11	3	8	6	5	7	9	8	8

Table 3. Comparison of haplotype diversity(H) and nucleotide diversity(π) in the Korea and North-Pacific Bering sea samples

		Korea		North-Pacific Bering sea						
		Nam.	S02	S05	S07	S11	S14	S17	S21	S25
#	Sequence	11	3	8	6	5	7	9	8	8
#	haplotype	3	3	5	5	4	6	3	5	2
	H	0.34545	1.0000	0.7857	0.9333	0.9000	0.9524	0.6667	0.8571	0.2500
	π	0.00099	0.0061	0.0047	0.0101	0.0073	0.0031	0.0027	0.0047	0.0005
	Sum(H)	0.34545				0.79315				
	Sum(π)	0.00099				0.00488				

Table 4. Genetic distance among each samples of Korea and North-Pacific Bering sea calculated by Kimura's two parameter method

	Korea	S02	S05	S07	S11	S14	S17	S21	S25
Korea	0.00099	0.00361	0.00051	0.00040	0.00070	0.00204	0.00069	0.00089	0.00298
S02	0.00715	0.00609	0.00147	0.00115	0.00098	0.00061	0.00128	0.00093	0.00061
S05	0.00336	0.00686	0.00470	-0.00036	-0.00025	0.00017	-0.00032	-0.00019	0.00079
S07	0.00598	0.00928	0.00708	0.01018	-0.00036	0.00007	-0.00024	0.00037	0.00064
S11	0.00486	0.00769	0.00576	0.00839	0.00732	-0.00006	-0.00028	-0.00039	0.00036
S14	0.00410	0.00522	0.00408	0.00672	0.00517	0.00313	0.00020	-0.00006	-0.00007
S17	0.00256	0.00569	0.00340	0.00622	0.00475	0.00313	0.00274	-0.00017	0.00066
S21	0.00373	0.00633	0.00451	0.00707	0.00562	0.00385	0.00355	0.00470	0.00033
S25	0.00371	0.00388	0.00337	0.00596	0.00424	0.00172	0.00226	0.00291	0.00046

Diagonal: Intrapopulational distance d_{ii}

Lower left: Interpopulational distance d_{ij}

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

Table 5. P values(upper diagonal) and χ^2 test(below diagonal) among Korea and North-Pacific Bering sea samples

	Korea	S02	S05	S07	S11	S14	S17	S21	S25
Korea	-	0.00202	0.02623	0.03443	0.07137	0.13633	0.07656	0.01838	0.10096
S02	0.00020*	-	0.00150	0.32394	0.42319	0.23810	0.44728	0.10928	0.30109
S05	0.02750	0.00010*	-	0.08624	0.48118	0.17842	0.47002	0.60426	0.48537
S07	0.00690*	0.57250	0.15010	-	0.32431	0.30596	0.54049	0.28084	0.49639
S11	0.01700*	1.00000	0.80200	0.28460*	-	0.17672	0.32107	0.08596	0.26922
S14	0.13790	0.46050	0.10390*	0.55310	0.06200*	-	0.02338	0.27823	0.60026
S17	0.01000*	1.00000	0.78480	1.00000	0.57650	0.00250*	-	0.24062	0.36234
S21	0.00710*	0.13540	0.58300*	0.32610	0.04900*	0.30190	0.18140*	-	0.15157

S25	0.03010*	0.50600	1.00000	1.00000	0.31760*	1.00000	0.48930	0.15250	-
-----	-----------------	---------	---------	---------	-----------------	---------	---------	---------	---

* Significant differences are shown (95% level of confidence)

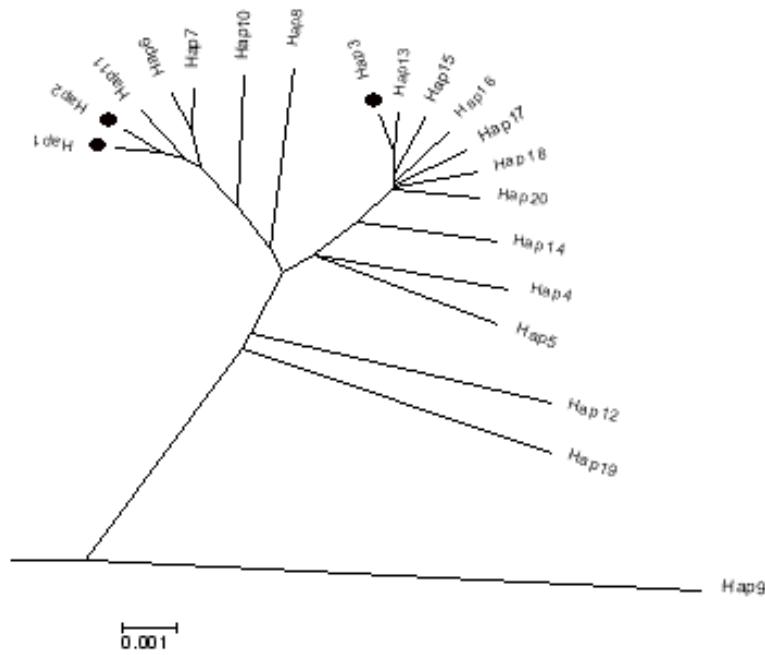


Fig. 1. Phylogenetic tree among 20 haplotypes revealed from Korea and North-Pacific Bering sea salmon samples based on mitochondrial control region sequences.

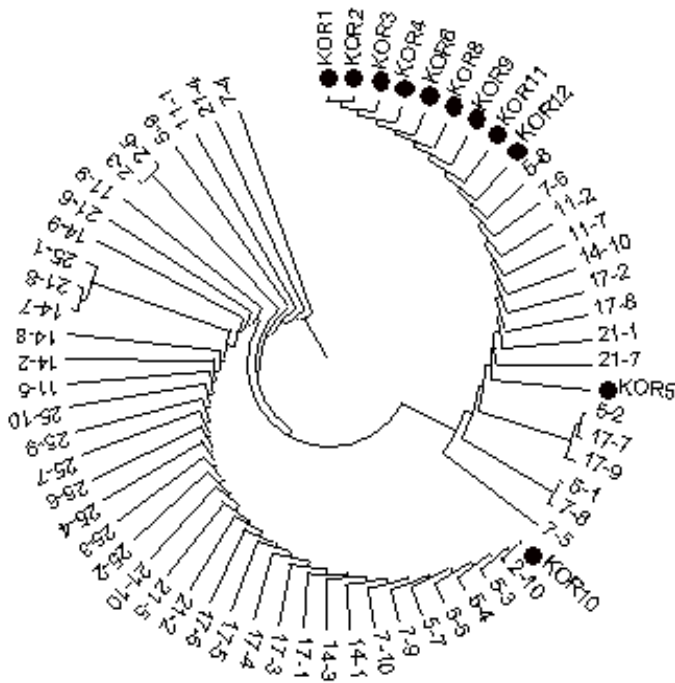


Fig. 2. Genetic relationship (topology) among 65 salmon samples revealed from Korea and North-Pacific Bering sea

based on mitochondrial control region sequences.