

Geographic variations of nuclear DNA on walleye pollock (preliminary results)

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Walleye pollock, *Theragra chalcogramma*, is one of the most important species in the North Pacific and Bering Sea ecosystems. However the amount of genetic population structuring of walleye pollock is uncertain. In the present study, the geographic variations of nuclear DNA on walleye pollock collected in spawning areas ranging from the Japan Sea to the Gulf of Alaska was investigated using SNP analysis (Table 1).

Intron regions between exon 5 and 6 of calmodulin genes were amplified using the polymerase chain reaction (PCR). PCR amplicons were treated with ExoSap-It (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) to degrade unincorporated primers and dNTPs and used as templates for SNP analysis. One oligonucleotide primer (SNP1, 5'-TCGTGGGCCTCAACGTTAAACTG-3') was designed for SNP analysis. This primer was subsequently labeled by a single base extension diagnostic for the SNP using the SNaPshot® Multiplex Kit (Applied Biosystems, Foster City, CA, USA) according to manufacture's protocols and then detected by electrophoresis using ABI3100 automated sequencer (Applied Biosystems). Genotypes were named S type (TG: 1copes) and L type (TG 2copes)(Fig. 1).

Genotypic conformance to Hardy-Weinberg equilibrium expectations were tested χ^2 test. Analysis of molecular variance (AMOVA) among regional groupings of samples was conducted using Arlequin ver 2.00 (Schneider et al. 2000). Nei's genetic distance were calculated using PYLIP program and was constructed UPGMA tree.

Composite of alleles frequencies were different between eastern and western Pacific and Bering Sea, and these results are concordant with previous studies using allozymes and mtDNA PCR-RFLP (Table 1, Figs. 2,5). There were significant differences in population pairwise (F_{st}) among some pairs (Table 3). From Nei's genetic distances (Table 2), the UPGMA tree was constructed and sampling regional groups were divided into three cluster (Fig. 3). Based upon these results, we estimated the possibility population structure of walleye pollock (Fig. 4).

Insertion	Sequence 1	Sequence 2	Sequence 3	Sequence 4	Sequence 5	Sequence 6	Sequence 7	Sequence 8	Position
insert7RN	TTATCTCTGG	CGGGTTAAC	GTTAAACTGA	TATAGTTATC	GTCCGGTGGT	TAACGTAAA	CGG		80
insert12R	TTATCTCTGG	CGGGTTAAC	GTTAAACTGA	TATAGTTATC	GTCCGGTGGT	TAACGTAAA	CGG		80
insert4R	TTATCTCTGG	CGGGTTAAC	GTTAAACTGA	TATAGTTATC	GTCCGGTGGT	TAACGTAAA	CGG		80
insert5R	TTATCTCTGG	CGGGTTAAC	GTTAAACTGA	TATAGTTATC	GTCCGGTGGT	TAACGTAAA	CGG		80
insert3R	TTATCTCTGG	CGGGTTAAC	GTTAAACTGA	TATAGTTATC	GTCCGGTGGT	TAACGTAAA	CGG		80
insert2R	TTATCTCTGG	CGGGTTAAC	GTTAAACTGA	TATAGTTATC	GTCCGGTGGT	TAACGTAAA	CGG		80
insert11RN	TTATCTCTGG	CGGGTTAAC	GTTAAACTGA	TATAGTTATC	GTCCGGTGGT	TAACGTAAA	CGG		80
insert9RN	TTATCTCTGG	CGGGTTAAC	GTTAAACTGA	TATAGTTATC	GTCCGGTGGT	TAACGTAAA	CGG		80
	*****	*****	*****	*****	*****	*****	*****		
insert7RN	CTCAGCGGTA	AAATGTGATA	TTTGTCAATG	GGTTGTTAAC	GTCAACCGTG	ATATTTATCG	TGGGCTCAA	CGTTAAACTG	160
insert12R	CTCAGCGGTA	AAATGTGATA	TTTGTCAATG	GGTTGTTAAC	GTCAACCGTG	ATATTTATCG	TGGGCTCAA	CGTTAAACTG	160
insert4R	CTCAGCGGTA	AAATGTGATA	TTTGTCAATG	GGTTGTTAAC	GTCAACCGTG	ATATTTATCG	TGGGCTCAA	CGTTAAACTG	160
insert5R	CTCAGCGGTA	AAATGTGATA	TTTGTCAATG	GGTTGTTAAC	GTCAACCGTG	ATATTTATCG	TGGGCTCAA	CGTTAAACTG	160
insert3R	CTCAGCGGTA	AAATGTGATA	TTTGTCAATG	GGTTGTTAAC	GTCAACCGTG	ATATTTATCG	TGGGCTCAA	CGTTAAACTG	160
insert2R	CTCAGCGGTA	AAATGTGATA	TTTGTCAATG	GGTTGTTAAC	GTCAACCGTG	ATATTTATCG	TGGGCTCAA	CGTTAAACTG	160
insert11RN	CTCAGCGGTA	AAATGTGATA	TTTGTCAATG	GGTTGTTAAC	GTCAACCGTG	ATATTTATCG	TGGGCTCAA	CGTTAAACTG	160
insert9RN	CTCAGCGGTA	AAATGTGATA	TTTGTCAATG	GGTTGTTAAC	GTCAACCGTG	ATATTTATCG	TGGGCTCAA	CGTTAAACTG	160
	*****	*****	*****	*****	*****	*****	*****		
insert7RN	ATATTTGT	TGCGTGGCTT	TCAAGTTTAA	ACCGTGATAT	TTATCGTCGG	TGGCCATCTT	GGAGCTCTAA	CGTCTGTGCT	238
insert12R	ATATTTGT	TGCGTGGCTT	TCAAGTTTAA	ACCGTGATAT	TTATCGTCGG	TGGCCATCTT	GGAGCTCTAA	CGTCTGTGCT	238
insert4R	ATATTTGT	TGCGTGGCTT	TCAAGTTTAA	ACCGTGATAT	TTATCGTCGG	TGGCCATCTT	GGAGCTCTAA	CGTCTGTGCT	238
insert5R	ATATTTGT	TGCGTGGCTT	TCAAGTTTAA	ACCGTGATAT	TTATCGTCGG	TGGCCATCTT	GGAGCTCTAA	CGTCTGTGCT	238
insert3R	ATATTTGT	TGCGTGGCTT	TCAAGTTTAA	ACCGTGATAT	TTATCGTCGG	TGGCCATCTT	GGAGCTCTAA	CGTCTGTGCT	238
insert2R	ATATTTGT	TGCGTGGCTT	TCAAGTTTAA	ACCGTGATAT	TTATCGTCGG	TGGCCATCTT	GGAGCTCTAA	CGTCTGTGCT	240
insert11RN	ATATTTGT	TGCGTGGCTT	TCAAGTTTAA	ACCGTGATAT	TTATCGTCGG	TGGCCATCTT	GGAGCTCTAA	CGTCTGTGCT	240
insert9RN	ATATTTGT	TGCGTGGCTT	TCAAGTTTAA	ACCGTGATAT	TTATCGTCGG	TGGCCATCTT	GGAGCTCTAA	CGTCTGTGCT	240
	*****	*****	*****	*****	*****	*****	*****		
insert7RN	GTGCTGTTGC	CAGGACGGAA	ACGGCTACAT	CAGTGCT					275
insert12R	GTGCTGTTGC	CAGGACGGAA	ACGGCTACAT	CAGTGCT					275
insert4R	GTGCTGTTGC	CAGGACGGAA	ACGGCTACAT	CAGTGCT					275
insert5R	GTGCTGTTGC	CAGGACGGAA	ACGGCTACAT	CAGTGCT					275
insert3R	GTGCTGTTGC	CAGGACGGAA	ACGGCTACAT	CAGTGCT					275
insert2R	GTGCTGTTGC	CAGGACGGAA	ACGGCTACAT	CAGTGCT					277
insert11RN	GTGCTGTTGC	CAGGACGGAA	ACGGCTACAT	CAGTGCT					277
insert9RN	GTGCTGTTGC	CAGGACGGAA	ACGGCTACAT	CAGTGCT					277
	*****	*****	*****	*****					

Fig. 1. Alignment results of sequence analysis of cDNA (Calmodulin gene).

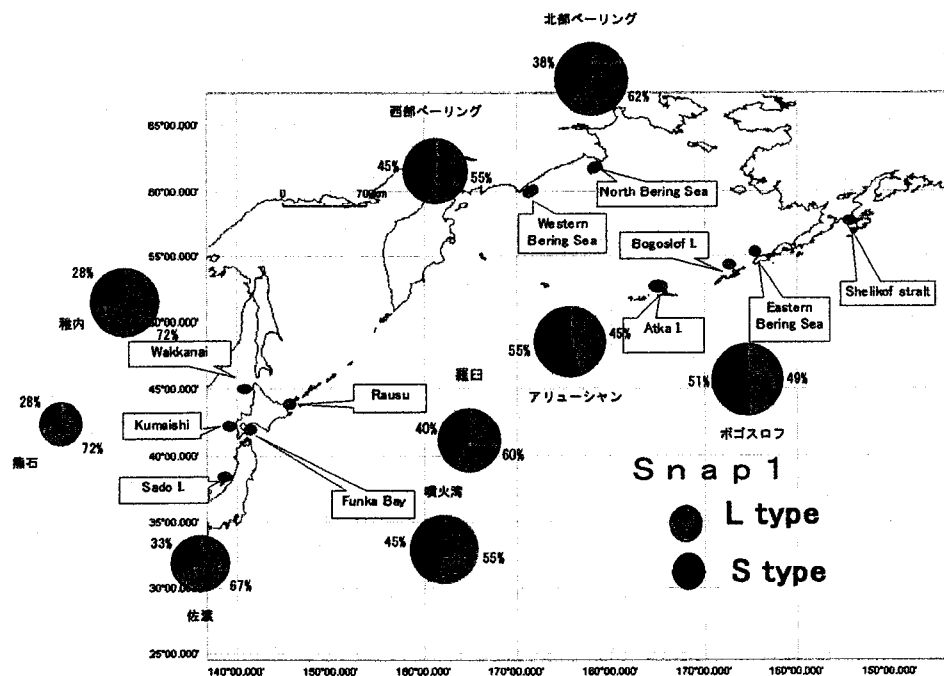


Fig. 2. The allele frequencies of L and S type obtained from SNAP analysis.

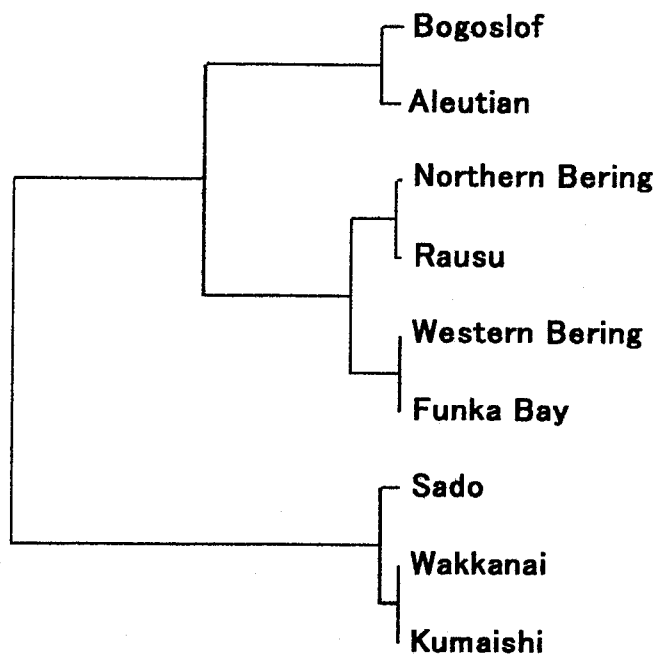


Fig. 3. UPGMA dendrogram of relationships among sampling sites of walleye pollock.

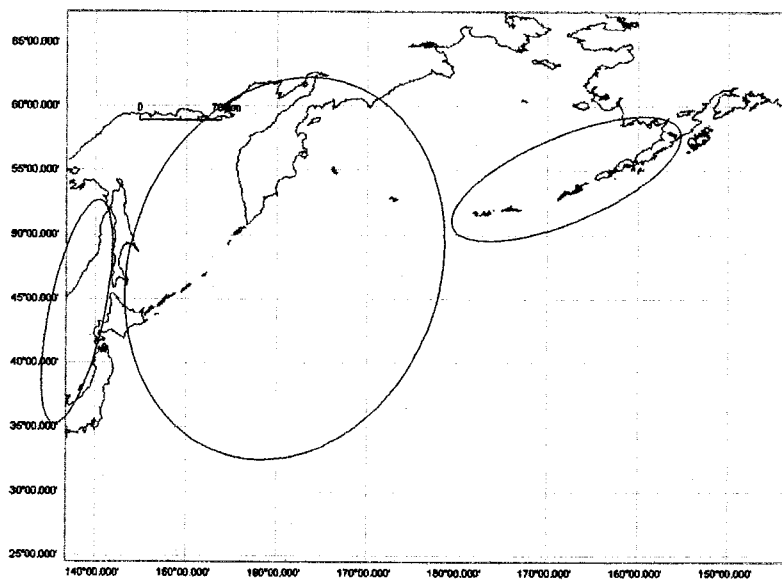


Fig. 4. Population structure of walleye pollock from this study.

A) SOD*

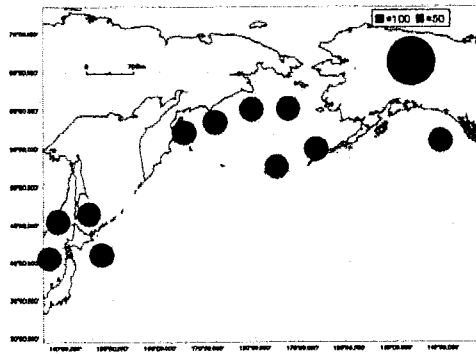
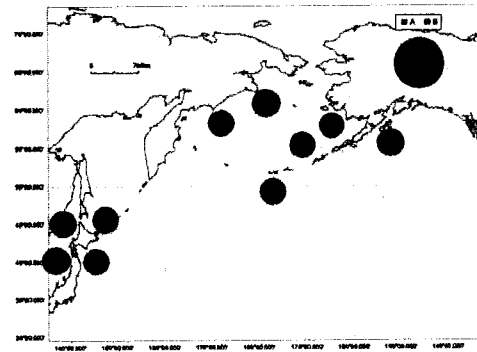
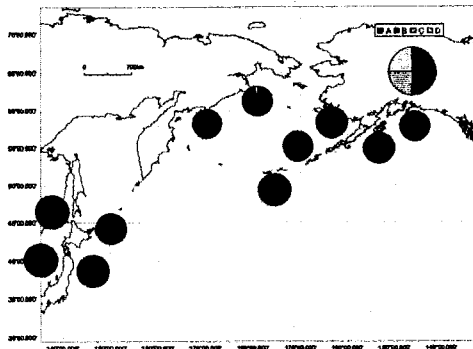
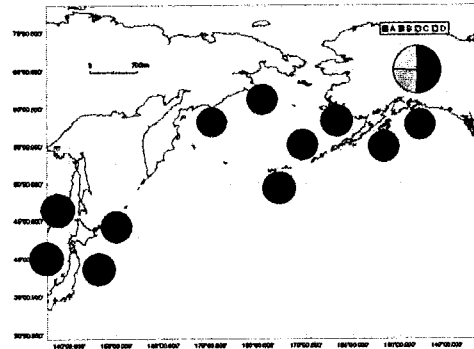
B) D-Loop region by *Hinf*IC) 1-16S rRNA region by *Msp*ID) NADH5-6 region by *Msp*I

Fig. 5. The results of allozyme analysis and PCR-RFLP analysis of mtDNA.

Table 1. Sample number, allele frequencies, χ^2 values of departure from Hardy-Weinberg's equilibrium.

Area	Number	Allele Frequencies		χ^2 Value
		S type	L type	
Bogoslof Area	40	0.51	0.488	0.103
Aleutian Area	39	0.551	0.449	1.438
Northern Bering Sea	33	0.379	0.621	5.835*
Western Bering Sea	39	0.449	0.551	0.552
Rausu	40	0.4	0.611	0.156
Wakkanai	37	0.284	0.716	0.000
Funka Bay	40	0.45	0.55	0.495
Kumaishi	50	0.28	0.72	0.574
Sado	40	0.325	0.675	1.637

*: significant differences ($p < 0.05$)

Table 2. Nei's genetic distance among sampling area.

	Bering Sea			Rausu	Wakkanai	Funka Bay	Kumaishi	Sado
	Aleutian Area	Northern Area	Western Area					
Bogoslof Area	0.0029	0.0351	0.0082	0.0252	0.0972	0.0079	0.1003	0.0673
Aleutian Area		0.0586	0.0208	0.0454	0.1358	0.0204	0.1396	0.0893
Northern Area			0.0092	0.0008	0.0146	0.0095	0.0158	0.0049
Western Area				0.0046	0.0476	0.0000	0.0498	0.0279
Rausu					0.0223	0.0048	0.0238	0.0097
Wakkanai						0.0482	0.0000	0.0025
Funka Bay							0.0504	0.0284
Kumaishi								0.003
Sado								

Table 3. The results of population pairwise Fst test.

	Bering Sea			Rausu	Wakkanai	Funka Bay	Kumaishi	Sado
	Aleutian Area	Northern Area	Western Area					
Bogoslof Area								
Aleutian Area								
Northern Area								
Western Area								
Rausu								
Wakkanai								
Funka Bay								
Kumaishi								
Sado								

Yellow pairs indicate significant differences ($p < 0.05$).