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

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Walleye pollock, *Theragra chalocogramma,* 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: 1cope) 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 (Fst) 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).

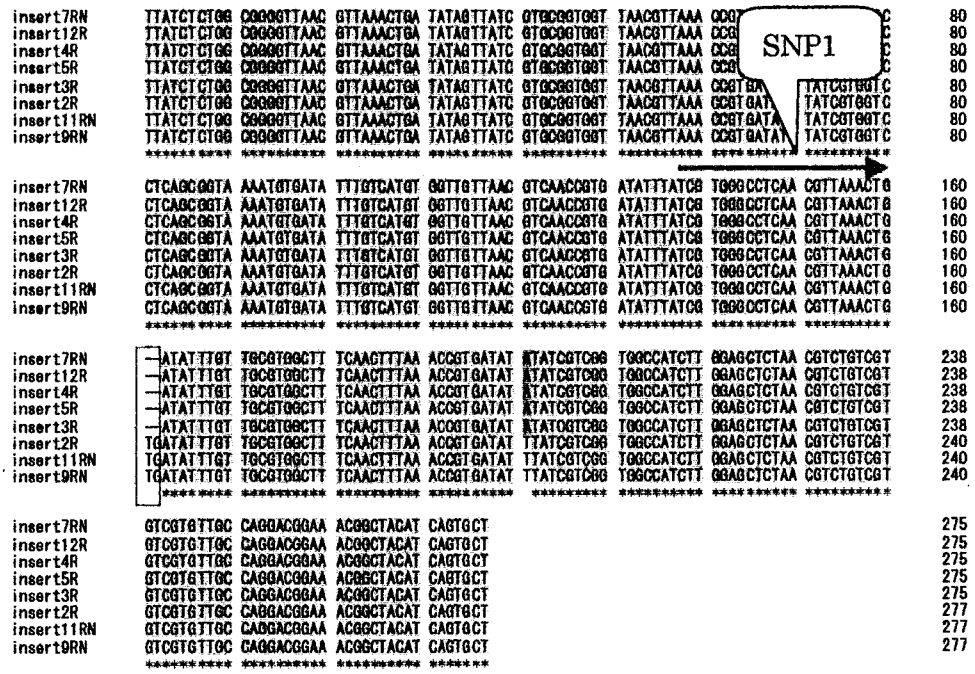


Fig. 1. Alignment results of sequence analysis of cDNA (Calmodulin gene).

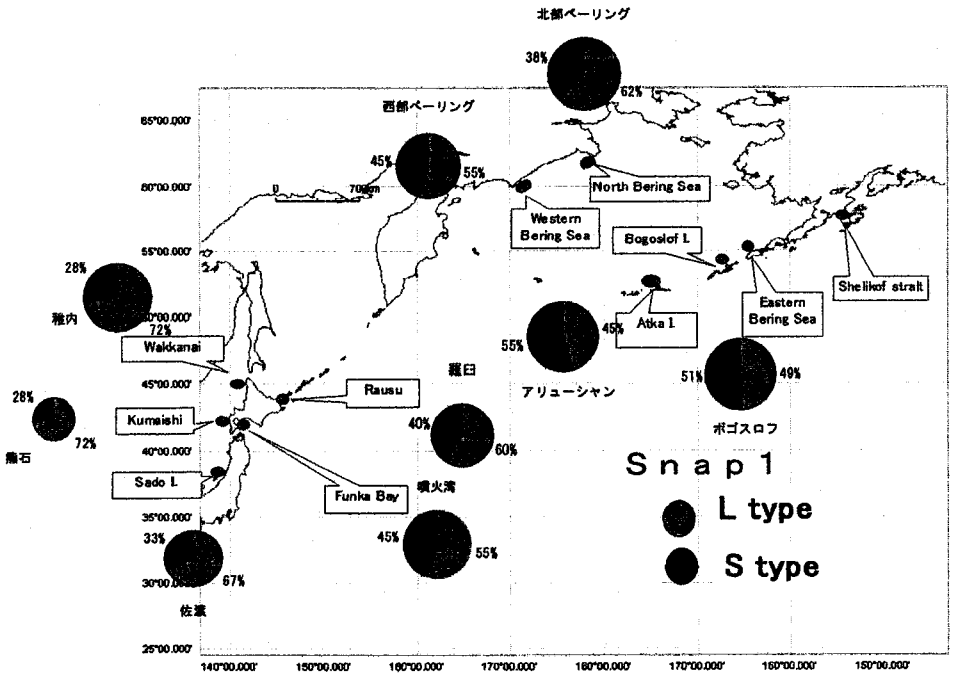


Fig. 2. The allele frequencies of L and S type obtained from SNAP analysis.

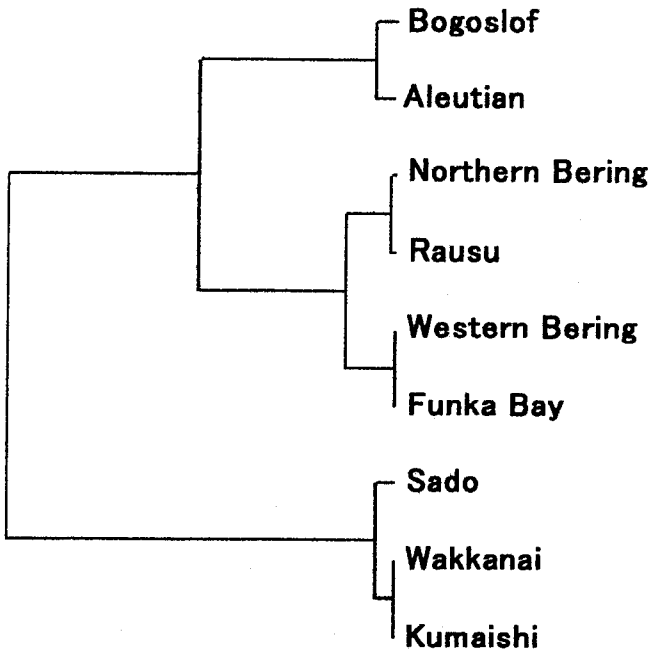


Fig. 3. UPGMA dendorogram of relationships among sampling sites of walleye pollock.

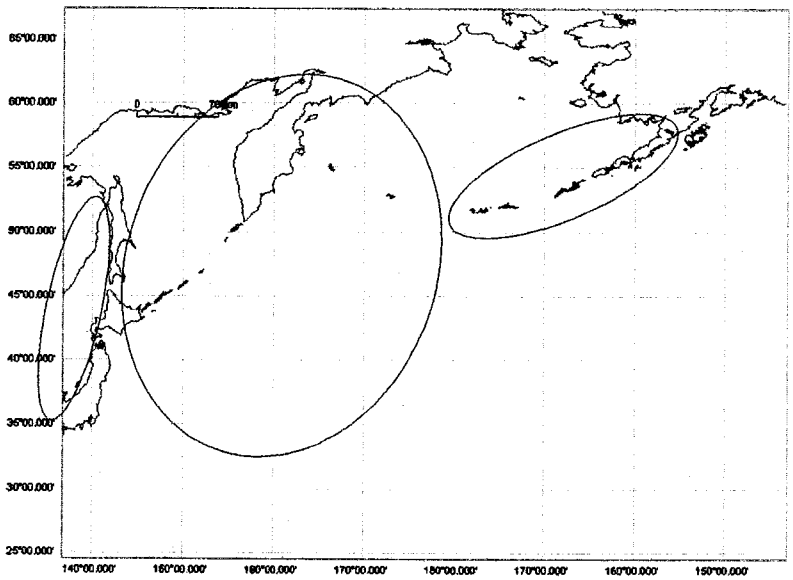
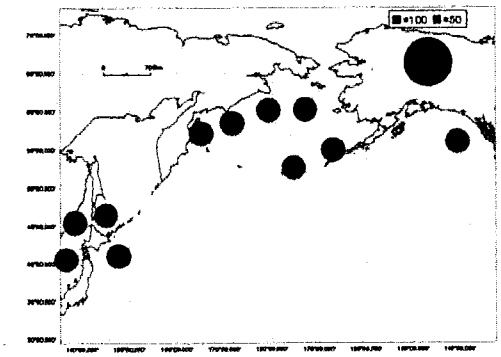
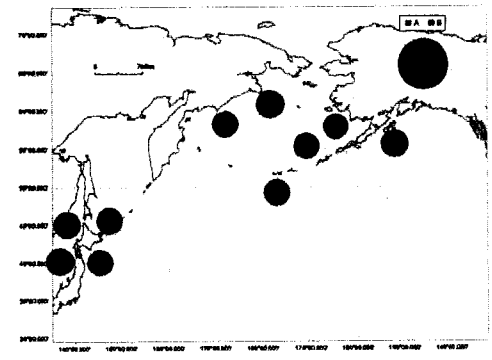


Fig. 4. Population structure of walleye pollock from this study.

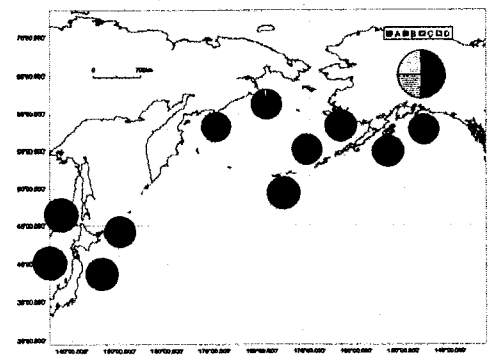
A) SOD*



B) D-Loop region by *Hinf*I



C) 1-16S rRNA region by *Msp*I



D) NADH5-6 region by *Msp*I

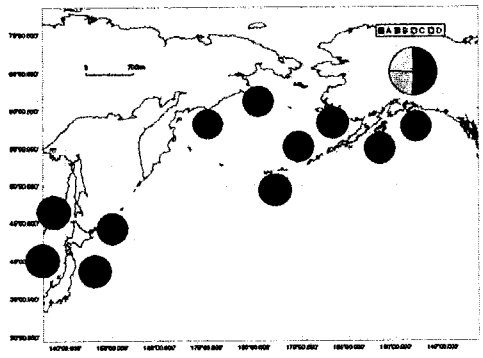


Fig. 5. The results of allozyme analysis and PCR-RFLP analysis of mtDNA.

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

		Allele Frequencyies		
Area	Number	S type	L type	χ^2 Value
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							
	Aleutian Area	Northern Area	Western Area	Rausu	Wakkanai	Funka Bay	Kumaishi	Sado
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	0.0993
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							
	Aleutian Area	Northern Area	Western Area	Rausu	Wakkanai	Funka Bay	Kumaishi	Sado
Bogoslof Area								
Aleutian Area								
Northern Area								
Western Area								
Rausu								
Wakkanai								
Funka Bay								
Kumaishi								
Sado								

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