Genetic Variation and Population Structure in Masu Salmon *Oncorhynchus masou* of Japan

Toshio Okazaki*

(Accepted February 28, 1986)

Genetic variation and population structure among masu salmon river populations in Japan were examined. Enzymatic proteins in tissue samples of fish among twenty populations collected from eighteen rivers were assayed for electrophoretically detectable variants. 34 loci were tentatively identified, 17 (50.0%) of which were polymorphic. Significant differences in the frequencies of alleles were observed even among the proximal river populations. The magnitude of genetic divergence among masu salmon river populations is relatively large among Pacific salmon, *Oncorhynchus* spp. While stability of allelic frequencies was observed between annual samples from same river systems. According to the resulting dendrogram, the examined populations appeared to be roughly divided into two geographic groups at the Shiretoko Peninsula of Hokkaido; (1) the rivers entering the Strait of Nemuro and the Pacific Ocean, (2) the rivers entering the Okhotsk Sea and the Japan Sea. The obtained level of the average heterozygosity of masu salmon was 5.2%.

Masu salmon *Oncorhynchus masou* is distributed exclusively in the Far East, making a striking contrast to the other species of Pacific salmon (*Oncorhynchus* spp.) which extends widely in the freshwater along the coast of the North Pacific Ocean. In Japan, masu salmon is distributed widely, centering around Hokkaido and the northern part of Honshu, however the spawning stocks of the anadromous population extend southerly no farther than the middle of Honshu.¹⁾

Some attempts have been made to find the population structure of masu salmon by using the tagging and morphological methods.²⁾ In addition to the conventional methods, numerous investigations based upon biochemical genetic methods have been applied to gain a better understanding of the genetic structure of salmonid populations in recent years.^{3,4)} However such studies in Japan have focused on chum salmon^{5,6)} *Oncorhynchus keta* instead of masu salmon. It is partly because most of the masu salmon population have been maintained naturally which lead to the difficulties of collecting specimens in addition to the actual sparse population. Therefore, the conclusion drawn from biochemical population genetics concerning masu salmon may exclude the significant effects of transplantation on the natural population.

This paper examines the biochemical population genetics of twenty populations of masu

salmon collected from eighteen rivers in Japan. The purpose of the present study is to define the genetic structure and maintenance of this species based upon the measurement of the amount of genetic differentiation among river populations using allele-frequency data of protein-coding loci. Its purpose also includes the documentation of allelic frequencies of the masu salmon populations of Japan for identifying racial components of mixtures of stocks taken on the sea.

In Japan, *Oncorhynchus rhodurus*, a closely related species to masu salmon is distributed in southwestern Honshu, Shikoku and a part of Kyushu. Since masu salmon and *O. rhodurus* have been treated as separate species in general,⁷⁾ none of the specimens of the latter were included in the current analysis.

Materials and Methods

Sample locations of eighteen rivers are identified in Fig. 1 and sample size and dates of collection are listed in Table 2.

The specimens collected in Hokkaido rivers were juvenile fish. Judging from their body size,⁸⁾ most of them seemed to be one-year-old fish. The collections of the Teshio River and the Huren River were taken from their tributaries, namely the Naka River and the Anebetsu River respectively. The remainings were collected from main stream of each river system.

* Far Seas Fisheries Research Laboratory, Fisheries Agency of Japan, Ordo, Shimizu 424, Japan (岡崎登志夫: 遠洋水産研究所).

Fig. 1. Map of Japan showing the rivers where masu salmon were sampled.

In Honshu, all of the specimens used in this study were from hatcheries. Their parents were anadromous fish which return to each river. The specimens collected in the Unozumai, Miomote and Shinano Rivers were one-year-old fish. The fish from the Oippe, Tone, Yoneshiro, Aka and Jinzu Rivers were two-year-olds. The Tone River population were the fish which were collected from the Agatsuma River, one of the tributaries of the Tone River system, and had been reared at the Koide Branch of Niigata Prefectural Freshwater Fish Experimental Station. The current specimens were the lineage of their third generation. The collections of the Yoneshiro River and the Shinano River were from fish hatched and reared at their tributary hatcheries, namely the Ani River and the Uono River respectively.

In the Shiribetsu and Jinzu Rivers, the specimens were collected from successive years, in 1982 and 1983.

Whole fish bodies were frozen immediately following collection until processed for electrophoresis. Horizontal starch gel electrophoresis was used to detect enzyme variants. Processing of samples and electrophoretic methods have been described elsewhere.⁹⁾ The following four buffer systems were used: (1) a discontinuous Tris-citric acid (gel pH 8.5), lithium hydroxide-boric acid (tray pH 8.5) buffer system described by Ridgway *et al.*;¹⁰⁾ (2) a Tris-boric acid-EDTA buffer (pH 8.5) described by Markert and Faulhaber;¹¹⁾ (3) an amine (N-(3-Aminopropyl)-morpholine) citrate buffer (pH 6.5) described by Clayton and

Tretiak;¹²⁾ and (4) an amine (N-(3-Aminopropyl)-diethanolamine) citrate buffer (pH 7.0) described by Numachi *et al.*¹³⁾ with slight modification based on the Clayton and Tretiak.¹²⁾ Staining procedures followed methods outlined in Harris and Hopkinson¹⁴⁾ and Allendorf *et al.*¹⁵⁾ A list of enzyme stains used, their abbreviations, Enzyme Commission number, the numbers of loci expressed and others are given in Table 1. Tissues of liver, skeletal muscle and eye ball were used for screening. Tissue of heart was not used in the current analysis due to its small volume. Locus and allelic nomenclatures follow Allendorf and Utter.¹⁶⁾

Nei's genetic distance (D)¹⁷⁾ measure was used to quantify the degree of similarity between populations. Heterozygosity (H) was calculated for each locus in a population as follows: $H = 1 - \sum_i p_i^2$, where n is the number of alleles at the locus and p_i is the frequency of the i^{th} allele in the population. The mean heterozygosity over all thirty-one loci provided the value of average heterozygosity (H) for each population.

Results

Electrophoretic Variation

The following nineteen enzyme systems were examined. In the absence of the breeding data, the Mendelian nature of the electrophoretic variants was inferred from the banding patterns on the gels under the following criteria: (1) Banding patterns had to be consistent with the known molecular structure of that protein. (2) When a gene is expressed in more than one tissue, variant phenotypes should be parallel among tissues.¹³⁾

1. Alcohol Dehydrogenase (ADH), 6-Phosphogluconate Dehydrogenase (6-PGD), Superoxide Dismutase (SOD)

A single polymorphic locus having three-banded heterozygotes and single-banded homozygotes characteristic of dimeric proteins was detected in each enzyme system (Fig. 2). It was interpreted from the result of two allelic products.

The ADH and SOD loci were visualized in liver exclusively. The 6-PGD locus was strongly expressed in skeletal muscle as well as liver extracts.

2. Esterase (EST)

Electrophoretic variants were observed in several tissues, but their genetic interpretation was not obtained.

3. α -Glycerophosphate Dehydrogenase

The most common phenotypes of α -GDH had

Table 1. Enzyme, their abbreviations, Enzyme Commission numbers and locus designations, and buffer-tissue combinations used in this study

Enzyme	Abbreviation	Enzyme Commission number	Locus designation (if multiple)	Buffer system* ¹		
				Eye	Liver	Muscle
Adenylate kinase	AK	2.7.4.3.				4
Alcohol dehydrogenase	ADH	1.1.1.1			4	
Aspartate aminotransferase	AAT	2.6.1.1.		3		
Creatine kinase	CK	2.7.3.2	1			1
			2			1
Esterase	EST* ²	3.1.1.1.	?		1	1, 3
Glyceraldehyde-phosphate dehydrogenase	GAPDH	1.2.1.12	1			4
			2			4
α -Glycerophosphate dehydrogenase	α -GDH	1.1.1.8	1			3, 4
			2			3, 4
Isocitrate dehydrogenase	IDH* ²	1.1.1.42	1?			4
			2, 3?		4	
Lactate dehydrogenase	LDH	1.1.1.27	1, 2			1, 3
			3			1
			4		1	
			5	3		
Malate dehydrogenase	MDH	1.1.1.37	1, 2		3, 4	
			3, 4			3, 4
Malic enzyme	ME	1.1.1.40	1			4
			2		4	
Peptidase (substrate; glycyl leucine)	GL	3.4.11	1	2		2
			2	2		2
Peptidase (substrate; leucylglycylglycine)	LGG	3.4.11		2		
Phosphoglucumutase	PGM	2.7.5.1	1			3, 4
			2			3, 4
			3		3, 4	
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44			4	4
Phosphoglucose isomerase	PGI	5.3.1.9	1			1
			2			1
			3	1		1
Phosphomannose isomerase	PMI	5.3.1.8		3		3
Sorbitol dehydrogenase	SDH	1.1.1.14	1		1	
			2		1	
Superoxide dismutase	SOD	1.15.1.1.			2	

*¹ See Materials and Methods for buffer designations.*² Variable phenotypes observed but genetic model uncertain.

three bands reflecting the gene products of two loci where the central band represented the heterodimeric product between the two loci as observed in some species of salmonid fishes.¹⁰⁾ Several five-banded phenotypes reflecting the products of three alleles were observed. Their banding patterns suggested that the variant alleles exist on the cathodal locus (α -GDH-1; Fig. 2).

4. Isocitrate Dehydrogenase (IDH)

Electrophoretic variants were observed upon staining for IDH in muscle extracts. However the resolution was not clear and the genetic nature of these observed variants is not known.

A lot of phenotypes including triple-banded

patterns with asymmetric banding intensities were found on the gels in liver extracts. These electrophoretic variants were assumed to be the products of two disomic loci as reported in chum salmon.²⁰⁾ They were presumably reflecting the products of several alleles at both loci. Their genetic model remains unclear.

5. Lactate Dehydrogenase (LDH)

Lactate dehydrogenase is a tetrameric enzyme encoded by five loci in salmon.²¹⁾ The duplicate loci, LDH-1 and LDH-2, are predominant in skeletal muscle. The heterodimeric products between the two loci appeared, although the electrophoretic resolution did not clearly show all

Fig. 2. Photographs and diagrammatic representations of electrophoretic gel banding patterns of masu salmon.

five bands. LDH-3 and LDH-4 were strongly expressed in skeletal muscle and liver extracts, respectively, while LDH-5 expressed exclusively in eyeball. Since LDH-3 and LDH-4 appeared to have similar mobilities on the gels, the electrophoretic resolution did not show heterotetrameric bands. Among the above five loci, rare five-banded heterozygotes were observed for the LDH-3 locus (Fig. 2).

6. Malate Dehydrogenase (MDH)

Two zones of banding patterns appeared in masu salmon. Supernatant MDH is a dimeric enzyme encoded by four loci in salmonids.²²⁾ It was appeared that one pair of duplicate loci, MDH-1,2, code for the least anodal zone of activity and are most strongly expressed in liver extracts.

Another duplicate loci pair, MDH-3,4, code for the more anodal form and are more strongly expressed in skeletal muscle. Single- and triple-banded phenotypes observed with asymmetric banding intensities support the two loci coding for each zone of banding patterns. The phenotypes observed for MDH-1,2 and MDH-3,4 were interpreted from the result of five and four allelic products, respectively (Fig. 2).

7. Malic Enzyme (ME)

Two zones of activity were observed for ME in muscle and liver extracts. The least anodal locus of ME (ME-1), which was best visualized in muscle, a slow broad-banded variant was seen in low frequencies. The electrophoretic patterns suggest a one-locus, two-allele model. It is also assumed to be tetrameric in masu salmon as observed in

Fig. 2. (Continued)

Three zones of banding patterns appeared to represent the products of three loci. PGM-1 migrated cathodally from the origin, while the

It was shown that three loci code for PGI in salmon²⁴⁾. The dimeric structure of this protein combined with the different electrophoretic mobilities of the common alleles for all three loci result in a fixed heterozygote effect with six bands being the common phenotype expressed in muscle extracts. The PGI-3 locus, the most anodal locus, was also expressed in eyeball. PGI-3 had a single-

Table 2. Sample size, collection date, allelic frequencies for each locus and level of average heterozygosity in river populations of masu salmon in Japan

Locus	Allele	Hokkaido							
		Tokushi-betsu R.	Shoko-tsu R.	Shari R.	Shibetsu R.	Nishi-betsu R.	Huren R.	Nozuka R.	Teshio R.
ADH	—100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	—40	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
α -GDH-1	—100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LDH-3	—180	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LGG	130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.743	0.670	0.549	0.629	0.667	0.828	0.636	0.711
MDH-1, 2	160	0.250	0.330	0.446	0.357	0.333	0.172	0.364	0.290
	60	0.007	0.000	0.005	0.014	0.000	0.000	0.000	0.000
MHD-3, 4	100	0.994	0.991	0.974	0.987	1.000	1.000	1.000	0.946
	140	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000
PGM-1	50	0.006	0.005	0.000	0.006	0.000	0.003	0.000	0.044
	10	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000
MHD-3, 4	130	0.000	0.005	0.008	0.000	0.000	0.000	0.000	0.011
	100	0.927	0.968	0.874	0.926	0.953	0.955	0.871	0.921*
PGM-1	80	0.073	0.032	0.126	0.074	0.047	0.045	0.129	0.080
	115	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM-1	70	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	—100	0.519	0.783	0.739	0.455	0.449	0.425	0.650	0.675
PGM-2	—160	0.375	0.196	0.194	0.523	0.455	0.560	0.300	0.225
	—120	0.106	0.022	0.061	0.023	0.096	0.015	0.050	0.075
PGM-2	—200	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.025
	100	1.000	0.991	0.985	1.000	0.995	1.000	1.000	1.000
6-PGD	700	0.000	0.009	0.016	0.000	0.005	0.000	0.000	0.000
	100	0.994	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGI-1	90	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000
PGI-2	50	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	150	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
PGI-3	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PMI	100	1.000	1.000	0.984	1.000	1.000	0.995	1.000	1.000
	90	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
SDH-1	110	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000
	100	0.966	0.991	0.954	1.000	1.000	0.950	1.000	1.000
SOD	90	0.000	0.009	0.035	0.000	0.000	0.050	0.000	0.000
	110	0.034	0.000	0.012	0.000	0.000	0.000	0.000	0.000
SOD	100	0.921	0.769	0.761	0.807	0.669	0.891	0.906	0.974
	210	0.079	0.231	0.239	0.194	0.332	0.109	0.094	0.026
SOD	290	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	1.000	1.000	1.000	1.000	1.000	1.000	0.986	1.000
SOD	60	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000
Collected date		Sep. 29 1983	Sep. 30 1983	Sep. 3 1982	Sep. 22 1983	Sep. 5 1982	Sep. 5 1982	Sep. 8 1982	Sep. 28 1983
Sample size		89	54	97	39	95	100	36	23
Average heterozygosity		0.047	0.043	0.063	0.053	0.053	0.041	0.051	0.046

* Significant deviations from Hardy-Weinberg distributions (χ^2 goodness of fit, $P < 0.05$).

Table 2. (Continued)

Hokkaido			Honshu								
Chitose R.	Shiri- betsu R. (1982)	Shiri- betsu R. (1983)	Oippe R.	Unozu- mai R.	Tone R.	Yone- shiro R.	Aka R.	Mi- omote R.	Shinano R.	Jinzu R. (1982)	Jinzu R. (1983)
0.995	1.000	1.000	0.960	1.000	0.974	0.979	1.000	0.991	0.890	0.965	0.944
0.006	0.000	0.000	0.040	0.000	0.026	0.021	0.000	0.009	0.110	0.035	0.056
0.984	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000	1.000
0.016	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.995	1.000	0.994	1.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.006	0.000
0.564	0.587	0.605	0.739	0.796	0.843	0.621	0.614	0.590	0.609	0.490	0.500
0.436	0.414	0.388	0.239	0.205	0.157	0.379	0.386	0.410	0.391	0.510	0.500
0.000	0.000	0.007	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.997	0.989	0.989	0.983	1.000	1.000	1.000	0.993	0.997	1.000	0.947*	0.981
0.000	0.009	0.010	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000
0.003	0.002	0.003	0.012	0.000	0.000	0.000	0.007	0.003	0.000	0.049	0.019
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.989	0.947*	0.977	0.965	0.956	0.981*	0.867	0.905	0.940	0.952	0.974	0.961
0.000	0.046	0.023	0.027	0.044	0.000	0.011	0.003	0.000	0.000	0.026	0.040
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000
0.011	0.007	0.000	0.007	0.000	0.019	0.122	0.093	0.060	0.048	0.000	0.000
0.563	0.662	0.696	0.490	0.533	0.430	0.817	0.609	0.781*	0.786	0.620	0.619
0.353	0.213	0.250	0.350	0.419	0.378	0.175	0.242	0.094	0.186	0.280	0.333
0.068	0.125	0.054	0.160	0.038	0.000	0.008	0.148	0.125	0.014	0.101	0.048
0.016	0.000	0.000	0.000	0.011	0.192	0.000	0.000	0.000	0.014	0.000	0.000
0.995	0.996	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.005	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.000	0.996	1.000	0.990	1.000	1.000	0.995	0.946	1.000	1.000	1.000	1.000
0.000	0.004	0.000	0.005	0.000	0.000	0.005	0.055	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.000	1.000	1.000	0.955	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.984	1.000	0.985	0.990	1.000	1.000	1.000	0.996	1.000	0.995	0.997	0.983
0.016	0.000	0.015	0.010	0.000	0.000	0.000	0.000	0.000	0.005	0.003	0.017
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
0.919*	0.919	0.982	0.865*	0.912	0.991	0.973	0.779	0.957	0.884	0.955	0.975
0.007	0.058	0.000	0.105	0.088	0.010	0.027	0.115	0.022	0.006	0.041	0.025
0.074	0.023	0.018	0.030	0.000	0.000	0.000	0.106	0.022	0.111	0.003	0.000
0.872	0.861	0.880	0.860	0.872*	0.789	0.850	0.804	0.735	0.774	0.805*	0.867
0.128	0.139	0.120	0.140	0.128	0.211	0.150	0.196	0.265	0.226	0.169	0.133
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000
1.000	1.000	1.000	0.980	1.000	1.000	1.000	1.000	0.962	1.000	0.936	0.983
0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.038	0.000	0.064	0.017
Sep. 20 1984	Aug. 8 1982	Sep. 27 1983	Apr. 12 1982	Apr. 23 1984	Aug. 23 1982	Aug. 31 1982	Sep. 6 1982	Mar. 12 1985	Mar. 11 1985	Oct. 27 1982	May-Oct. 1983
95	115	100	101	102	105	95	110	100	100	172	40
0.050	0.053	0.043	0.061	0.046	0.044	0.052	0.071	0.054	0.057	0.063	0.054

Table 3. Estimates of genetic distance among twenty populations of masu salmon in Japan based

	Sho- kotsu R.	Shari R.	Shi- betsu R.	Nishi- betsu R.	Huren R.	Nozuka R.	Teshio R.	Chitose R.	Shiri- betsu (1982) R.
Tokushibetsu R.	.00296	.00383	.00146	.00266	.00124	.00111	.00113	.00168	.00193
Shokotsu R.		.00116	.00385	.00340	.00581	.00181	.00201	.00226	.00110
Shari R.			.00385	.00390	.00762	.00140	.00280	.00253	.00102
Shibetsu R.				.00091	.00167	.00207	.00365	.00162	.00296
Nishibetsu R.					.00294	.00355	.00526	.00254	.00346
Huren R.						.00382	.00403	.00372	.00522
Nozuka R.							.00080	.00167	.00101
Teshio R.								.00232	.00138
Chitose R.									.00082
Shiribetsu R. (1982)									
Shiribetsu R. (1983)									
Oippe R.									
Unozumai R.									
Tone R.									
Yoneshiro R.									
Aka R.									
Miomote R.									
Shinano R.									
Jinzu R. (1982)									

and triple-banded phenotypes which reflected homozygotes and heterozygotes of three alleles. Rare variants were observed at the remaining loci. The observed banding patterns suggested that these phenotypes reflect the products of two and three alleles, respectively (Fig. 2).

11. Phosphomannose Isomerase (PMI)

A single zone of activity having single- and double-banded phenotypes reflecting the products of three alleles was observed for this monomeric enzyme. PMI was most expressed in skeletal muscle.

12. Sorbitol Dehydrogenase (SDH)

SDH was shown to have a tetrameric structure in vertebrates.²⁵⁾ It is also assumed to be tetrameric in masu salmon, although the electrophoretic resolution did not clearly show all five heterotetrameric bands. Distinctive variation was observed in liver extracts. The most common phenotype of SDH had three or four bands probably reflecting the gene products of two loci. Including asymmetric banding patterns, single- and five-banded phenotypes were also observed. These phenotypes are best interpreted by reflecting the products of three alleles. The banding patterns observed suggested that the variant alleles exist on the least anodal locus (Fig. 2).

13. Adenylate Kinase (AK), Aspartate Amino-transferase (AAT), Creatine Kinase (CK), Glyceraldehyde-phosphate Dehydrogenase (GAPDH),

Peptidase (substrate; glycyl leucine: GL)

These enzymes expressed no variant forms.

Ak was visualized with extracts of skeletal muscle and was all represented by a single invariant band.

AAT was expressed in eyeball and skeletal muscle. AAT is a dimeric enzyme encoded by two loci in heart and skeletal muscle, and one locus in eyeball in salmon.²⁴⁾ In the current analysis, a single invariant band was observed in eyeball, although clear resolution was not observed in skeletal muscle.

CK, expressed by four-banded invariant form in all specimens, is encoded by two loci.²⁶⁾

GAPDH and GL were represented by two recognizable forms with different electrophoretic mobility. No variants were observed in masu salmon. It is assumed that each is coded for by two loci.

Population Structures

Allelic frequencies of twenty masu salmon populations collected from Japanese rivers are presented in Table 2. At most of the polymorphic loci, the deviation of observed number from the expectation was not significant (assuming Hardy-Weinberg equilibrium; Table 2).

Many differences among populations are evident from comparisons of allelic frequencies at the polymorphic loci. However no alleles which

on gene frequencies at thirty-one loci

Shiribetsu R. (1983)	Oippe R.	Unozumai R.	Tone R.	Yoneshiro R.	Aka R.	Miomote R.	Shinano R.	Jinzu R. (1982)	Jinzu R. (1983)
.00173	.00081	.00054	.00220	.00387	.00297	.00490	.00425	.00345	.00266
.00077	.00325	.00302	.00440	.00122	.00259	.00087	.00105	.00220	.00223
.00143	.00439	.00460	.00680	.00163	.00246	.00126	.00177	.00168	.00174
.00267	.00218	.00164	.00280	.00520	.00387	.00582	.00505	.00285	.00208
.00347	.00248	.00242	.00256	.00582	.00377	.00479	.00480	.00333	.00325
.00462	.00172	.00064	.00173	.00746	.00562	.00906	.00753	.00651	.00534
.00091	.00252	.00213	.00451	.00192	.00283	.00310	.00291	.00216	.00142
.00104	.00236	.00212	.00427	.00224	.00340	.00356	.00334	.00305	.00249
.00071	.00186	.00225	.00400	.00294	.00170	.00298	.00229	.00096	.00057
.00033	.00202	.00265	.00495	.00152	.00114	.00130	.00151	.00090	.00087
	.00231	.00239	.00435	.00138	.00205	.00161	.00145	.00109	.00077
		.00064	.00216	.00455	.00205	.00455	.00393	.00331	.00308
			.00142	.00440	.00310	.00541	.00441	.00425	.00350
				.00647	.00524	.00683	.00587	.00623	.00565
					.00234	.00123	.00128	.00287	.00255
						.00209	.00198	.00233	.00255
							.00106	.00204	.00272
								.00227	.00221
									.00038

characterize specific river populations located in a given area were not observed through the current analysis. Similarly, geographic gradient of allelic frequencies was not appeared at any loci.

From the result of t test, highly significant differences were observed among the examined populations at most polymorphic loci. For example, significant differences were observed in 150 combinations out of 190 combinations at the PGM-1 locus. Furthermore, the greater part of them were significant at the 1% level.

On the other hand, no significant differences were observed between the two yearly collections from the Shiribetsu and Jinzu Rivers, respectively. This indicates the stability of allelic frequencies over generations within same river system.

Variants of loci for the same protein resulting in bands of the same mobility (*i.e.*, duplicated loci-MDH-1,2, MDH-3,4), were impossible to arbitrarily assign to a particular locus. In the following analysis, therefore, it was assumed that variants of duplicated loci occurred at an equal frequency at both loci.²⁷⁾

1. Genetic Variability

34 loci were tentatively identified (excepting EST, IDH), 17 loci (50.0%) of them were polymorphic when a locus is considered polymorphic in populations in which the frequency of the most common allele was less than 0.99.

Table 2 provides the average heterozygosity for

all populations. These values ranged from 4.1% to 7.1%. The unweighted mean value of average heterozygosity of twenty masu salmon populations was 5.2%.

2. Genetic Distance

Nei's genetic distance¹⁷⁾ among examined twenty populations was calculated using 31 loci (Table 3). The obtained values ranged from 0.0003 to 0.0091 with the average of 0.0028. Based upon the above, a dendrogram was constructed according to unweighted pair-group method²⁸⁾ (Fig. 3). A clear structuring of populations that conforms to their geographic locations was not evident. But the examined populations appeared to be roughly divided into two geographic groups at the Shiretoko Peninsula of Hokkaido except for a few river populations; (1) the rivers entering the Strait of Nemuro and the Pacific Ocean, (2) the rivers entering the Okhotsk Sea and the Japan Sea.

Discussion

In the northern populations of masu salmon the typically anadromous groups predominate, whereas in the southern populations they tend to remain freshwater without catadromous migration and they are destined to mature in freshwater. These traits occur strongly in males than in females. In Japanese rivers, therefore, females are predominant among the anadromous populations.²⁾

Fig. 3. Dendrogram drawn from indices of genetic distance among twenty populations of masu salmon in Japan.

HK; Hokkaido, HN; Honshu.

In the current analysis, all of the specimens collected in Hokkaido were juveniles instead of anadromous parental fish due to the difficulty of collecting specimens. Therefore, the author could not identify the specimens as anadromous or non-anadromous forms. However, it is considered that genetic isolation between them is not tenable from the facts that (1) anadromous females and non-migratory males spawn together,²⁹⁾ (2) non-migratory populations have arisen from anadromous forms independently in many rivers,³⁰⁾ (3) fish culture work has demonstrated that the non-migratory forms may turn into the migratory forms and vice versa.^{31, 32)}

All of the specimens collected in Honshu, were hatchery stocks. In a few populations, the number of parental fish used for the artificial spawning was small. Furthermore, the Tone River population used was the lineage of the hatchery stocks. Some of the above might bias the true feature of the population structure in Honshu.

The obtained allelic frequencies varied among the examined populations, although a loose structuring of populations that conforms to their geographic locations was observed (Fig. 3).

The anadromous populations of masu salmon originating in Japan move northward following their catadromous migration along the coast towards the Okhotsk Sea which is their main feeding areas in summer. Afterwards they turn to move southward to approach their wintering waters. Following the wintering, all fish migrate to approach their coastal areas of their origin.²⁾ The observed clustering of the masu salmon populations is presumably related to their migra-

tion patterns in the ocean. It is because proximal river populations of salmonid fishes show distinct distribution and migration patterns during their ocean phase.³³⁾ Since the obtained migration patterns of masu salmon remain sketchy, this kind of question remains to be solved in the future.

It is considered that masu salmon is a primitive species among the genus *Oncorhynchus*.³⁴⁾ This species is morphologically³⁵⁾ and genetically^{18, 36)} more closely related to the genus *Salmo* than all the existing species of *Oncorhynchus*. According to the obtained level of the average heterozygosity, the genetic variability of masu salmon is similar to that of *Oncorhynchus*.¹⁸⁾

The obtained levels of the genetic distance suggest that relatively high genetic divergence occurs among masu salmon river populations. In particular, significant differences in allelic frequencies were also observed among proximal river populations. These traits contrast well with that of chum salmon river populations which exhibit highly genetic similarity among proximal river populations.³⁷⁾ The current collections in Hokkaido rivers were the fish from wild stocks. When comparing the genetic distance of chum salmon to that of masu salmon in Hokkaido rivers, the latter ($D=0.0025$) is about four times as large as the former ($D=0.0006$).²⁰⁾ The levels of the genetic distance among the masu salmon populations in Hokkaido correspond to the levels obtained among wide ranged North American populations of chum salmon in Alaska, British Columbia and Washington.⁹⁾ The above suggests that each river population of masu salmon has its own particular genetic traits probably due to more precise homing ability than chum salmon.

On the other hand, the stability of allelic frequencies was observed between annual samples from same river systems (*i.e.*, the Shiribetsu River and the Jinzu River; Table 2). This indicates the usefulness of electrophoretic data for defining populations and estimating component populations of mixed fisheries.

With respect to the genetic divergence among tributary populations within a single river system, considerably differences were observed in the frequencies of alleles in sockeye salmon *Oncorhynchus nerka*.³⁸⁾ Although this kind of problems could not be examined in the current study, similar phenomena may occur in masu salmon populations.

The endemic range of masu salmon extends from western Kamchatka and the Amur River in north to the southeastern areas of the Korea Peninsula in south. The center of its distribution is the rivers which enter the Okhotsk Sea and the Japan Sea.^{1,2)} Therefore, it is considered that many populations originated in various regions are intermingling complicatedly during the ocean phase. Since the Japan Sea is a main fishing ground for masu salmon, it is urgently requested to estimate racial components of mixed fisheries in the ocean. Biochemical genetic methods have provided valuable and unique insights concerning the estimating proportions of mixed populations.^{38,39)} However, there has been no information on the allelic frequencies of masu salmon populations in the rivers of the Soviet Union and Korea.

In future, the complete picture of the population structure of masu salmon throughout its range as well as the distribution, migration and component populations of mixed fisheries will gradually emerge with the accumulation of gene frequency data in other countries.

Acknowledgments

I would like to express my sincere gratitude to Messrs. H. Mayama, T. Nomura and K. Ohkuma, Hokkaido Salmon Hatchery, for their good offices for sampling in Hokkaido. Thanks are due to many generous people for their help in the sample collections; Mr. F. Kato of Seikai Regional Fisheries Research Laboratory, Fisheries Agency of Japan; Mr. T. Harako of Aomori Prefectural Freshwater Fish Experimental Station; Messrs. S. Koyama and K. Amita of Niigata Prefectural Freshwater Fish Experimental Station; Mr. H. Sugiyama of Akita Prefectural Freshwater Fish

Experimental Station; Mr. T. Kon-no of Yamagata Prefectural Freshwater Fish Experimental Station; Mr. Y. Tsuno of Fisheries Experimental Station of Toyama Prefecture.

I wish to express many thanks to Mr. K. Takagi of the Far Seas Fisheries Research Laboratory, Fisheries Agency of Japan, for his interest and encouragement.

References

- 1) S. Tanaka: *Bull. Intern. North Pacific Fish. Comm.*, **16**, 75-135 (1965).
- 2) S. Machidori and F. Kato: *Bull. Intern. North Pacific Fish. Comm.*, **43**, 1-138 (1984).
- 3) F. M. Utter, H. O. Hodgins, and F. W. Allendorf: *Biochemical and Biophysical Perspectives in Marine Biology*, Vol. 1, Academic Press, London, New York, 1974, pp. 213-237.
- 4) F. M. Utter, D. Campton, S. Grant, G. Milner, J. Seeb, and L. Wishard: in "Salmonid Ecosystems of the North Pacific" (ed. by D. C. Himsworth and W. J. McNeil), Oregon State Univ. Press, Corvallis, Oregon, 1980, pp. 285-304.
- 5) A. Kijima and Y. Fujio: *Bull. Japan. Soc. Sci. Fish.*, **45**, 287-295 (1979).
- 6) T. Okazaki: *Bull. Japan. Soc. Sci. Fish.*, **47**, 507-514 (1981).
- 7) M. Ohshima: Studies on the Dimorphic Salmons, *Oncorhynchus masou* (Brevoort) and *Oncorhynchus rhodurus* JORDAN and McGREGOR, Found in Japan and Adjacent Territories, Nire-Shobo, Sapporo, 1957, pp. 1-79.
- 8) H. Mayama, K. Ohkuma, and T. Nomura: *Marine Ranching Program Prog. Rep. on Masu Salmon Produc.*, Hokkaido Salmon Hatchery, **2**, 60-69 (1982).
- 9) B. May, J. E. Wright, and M. Stoneking: *J. Fish. Res. Bd. Can.*, **36**, 1114-1128 (1979).
- 10) G. J. Ridgway, S. U. Sherburne, and R. D. Lewis: *Trans. Am. Fish. Soc.*, **99**, 147-151 (1970).
- 11) C. L. Markert and I. Faulhaber: *J. Exp. Zool.*, **159**, 319-332 (1965).
- 12) J. W. Clayton and D. N. Tretiak: *J. Fish. Res. Bd. Can.*, **29**, 1169-1172 (1972).
- 13) K. Numachi, S. Nagahora, and I. Iwata: *Rep. Otsuchi Mar. Res. Cen.*, Univ. Tokyo, **5**, 87-95 (1979).
- 14) H. Harris and D. A. Hopkinson: *Handbook of Enzyme Electrophoresis in Human Genetics*, North Holland Publ. Co., Amsterdam, 1976.
- 15) F. W. Allendorf, N. Mitchell, N. Ryman, and G. Ståhl: *Hereditas*, **86**, 179-190 (1977).
- 16) F. W. Allendorf and F. M. Utter: in "Fish Physiology" (ed. by W. S. Hoar, D. S. Randall, and J. R. Brett), Academic Press, New York, 1979, pp. 407-454.
- 17) M. Nei: *Amer. Nat.*, **106**, 283-292 (1972).

- 18) W. S. Grant, R. Bakkala, F. M. Utter, D. J. Teel, and T. Kobayashi: *Fish. Bull.*, **81**, 667-677 (1983).
- 19) T. Okazaki: Intra-inter Specific Variations of Enzymes and Phylogenetic Study of Salmonid Fishes, M. S. Thesis, Univ. Tokyo, Tokyo, 1974, pp. 1-81.
- 20) T. Okazaki: *Bull. Far Seas. Fish. Res. Lab.*, **19**, 25-116 (1982).
- 21) F. M. Utter, F. W. Allendorf, and H. O. Hodgins: *Syst. Zool.*, **22**, 257-270 (1973).
- 22) G. S. Bailey, A. C. Wilson, J. E. Halver, and C. L. Johnson: *J. Biol. Chem.*, **245**, 5927-5940 (1970).
- 23) S. Povey, D. E. Wilson, H. Harris, I. P. Gormley, P. Perry, and K. E. Buckton: *Ann. Hum. Genet.*, **39**, 203-212 (1975).
- 24) B. May: Electrophoretic Variation in the Genus *Oncorhynchus*, M. S. Thesis, Univ. Washington, Seattle, 1979, pp. 1-96.
- 25) J. Op't Hof, U. Wolf, and W. Krone: *Human-genetik*, **8**, 178-182 (1969).
- 26) F. M. Utter, F. W. Allendorf, and B. May: *Biochem. Genet.*, **17**, 1079-1091 (1979).
- 27) F. W. Allendorf, F. M. Utter, and B. May: in "Isozymes IV: Genetics and Evolution" (ed. by C. L. Markert), Academic Press, New York, 1975, pp. 415-432.
- 28) P. H. Sneath and R. R. Sokal: *Numerical Taxonomy*, Freeman, San Francisco, 1973, pp. 188-308.
- 29) H. Utoh: *Freshwater Fishes*, **4**, 136-139 (1978).
- 30) H. Utoh: *Bull. Fac. Fish. Hokkaido Univ.*, **26**, 321-326 (1976).
- 31) S. Fujita: *J. Rakusui-kai*, **28**, 30-32 (1933).
- 32) M. Saguri: *J. Fish Culture Assoc.*, **6**, 35-40 (1936).
- 33) W. F. Royce, L. S. Smith, and A. C. Hartt: *Fish. Bull.*, **66**, 441-462 (1968).
- 34) F. Neave: *Trans. Roy. Soc. Can.*, **52**(III-5), 25-39 (1958).
- 35) R. J. Behnke, T. P. Koh, and P. R. Needham: *Copeia*, **1962**, 400-407 (1962).
- 36) K. Numachi: in "Biochemical Distinction of Fish Populations" (ed. by Japan. Soc. Sci. Fish.), Koseisha-Koseikaku, Tokyo, 1975, pp. 49-64.
- 37) T. Okazaki: *Bull. Japan. Soc. Sci. Fish.*, **49**, 189-196 (1983).
- 38) W. S. Grant, G. B. Milner, P. Krasnowski, and F. M. Utter: *Can. J. Fish. Aquat. Sci.*, **37**, 1236-1247 (1980).
- 39) T. D. Beacham, R. E. Withler, and A. P. Gould: *Can. J. Fish. Aquat. Sci.*, **42**, 437-448 (1985).