Russian Journal of Genetics, Vol. 38, No. 9, 2002, pp. 1066–1075. Translated from Genetika, Vol. 38, No. 9, 2002, pp. 1259–1269.

ANIMAL GENETICS

Allozyme Diversity and Genetic Divergence of the Dolly Varden *Salvelinus malma* Walbaum from the Kuril Islands

V. T. Omelchenko[1], E. A. Salmenkova[2], and S. V. Shedko[3]

[1] *Institute of Marine Biology, Russian Academy of Sciences, Vladivostok, 690041 Russia; fax: (4232) 31-09-00; e-mail: inmarbio@mail.primorye.ru*
[2] *Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, 119991 Russia; fax: (095) 132-89-62; e-mail: salm@vigg.ru*
[3] *Institute of Biology and Soil Sciences, Russian Academy of Sciences, Vladivostok, 690022 Russia; fax: (4232) 31-01-93; e-mail: shedko@ibss.dvo.ru*

Received January 8, 2002

Abstract—Genetic variation was studied in the southern subspecies of the Asian Dolly Varden *Salvelinus malma krascheninnikovi* from the Kuril Islands. Thirty-six genetic loci controlling 19 enzyme systems were analyzed in 13 Dolly Varden populations from the Shumshu, Paramushir, Onekotan, Rasshua, Simushir, Urup, Iturup, and Kunashir islands. In the studied populations, the proportion of polymorphic loci was 35 to 85% and the mean heterozygosity was 0.104 to 0.173; populations from the Kunashir Island were characterized by maximum heterozygosity. In the island populations examined, significant interpopulation heterogeneity of allele frequencies was found for all studied population pairs. For the all island populations, the interpopulation diversity (G_{ST} = 0.188) was comparable to this parameter for the populations from the Kunashir Island only (G_{ST} = 0.170). Genetic distances between populations did not correlate with the corresponding geographical distances, which indicates the lack of a pronounced gene exchange between the island populations. Cluster analysis and multidimensional scaling based on genetic distances did not reveal clear groups among the studied populations but indicated greater similarity within the Iturup–Simushir–Urup–Paramushir group and a greater genetic divergence of the Kunashir, Onekotan, Rasshua, and especially Shumshu populations. In the Shumshu population, allele frequencies indicate the admixture of genes of the northern Dolly Varden. The observed pattern of genetic differentiation was probably caused largely by genetic drift under the conditions of a limited gene flow because of homing (which is typical of the Dolly Varden) and the presence of isolated nonanadromous populations. The population–genetic analysis of the Dolly Varden from the Kuril Islands does not give grounds to distinguish any other isolated char species in this region than *S. malma*, which is represented by the southern form *S. m. krascheninnikovi* with an admixture of the northern form *S. m. malma* in the Shumshu Island.

INTRODUCTION

As a result of a long discussion on the taxonomic status of one of the most widespread chars, the Pacific Dolly Varden *Salvelinus malma* Walbaum (Pisces: Salmonidae), its status of an isolated species became generally recognized by the late 1990s [1]. To some extent, this was promoted by studies using karyological and biochemical–genetic methods [2–8].

However, in spite of reaching the consensus on the species status of Dolly Varden, the problem has not been settled. Some authors think reasonable to rank the brook Dolly Varden form *Salvelinus malma* morpha *curilis* (Pallas) as an isolated species [9] and to give the species status to the northern and southern forms (subspecies) of Dolly Varden (*Salvelinus malma malma* and *Salvelinus malma krascheninnikovi*, respectively), which have marked karyological differences [8]. Therefore, it is interesting to study genetic variation of *S. malma* populations from regions of sympatry of these forms. These regions include the Kuril Islands, especially their northern region, as ichthyological data indicate that the boundary of the Asian range of these forms lies between the Kamchatka Peninsula and the northern Kuril Islands [10].

The objectives of our work were the following: first, to study the genetic structure of Dolly Varden populations from the Kuril Islands in order to estimate interpopulation heterogeneity and taxonomic homogeneity in the studied part of range; second, based on the data on genetic variation in the examined populations, to make an attempt to reveal the area of intergradation of the northern and southern Dolly Varden, for which significant differences were found in allele frequencies of some genes [11–15].

MATERIALS AND METHODS

Fish was sampled in rivers of seven major islands of the Kuril Archipelago in 1999 and 2000 during the

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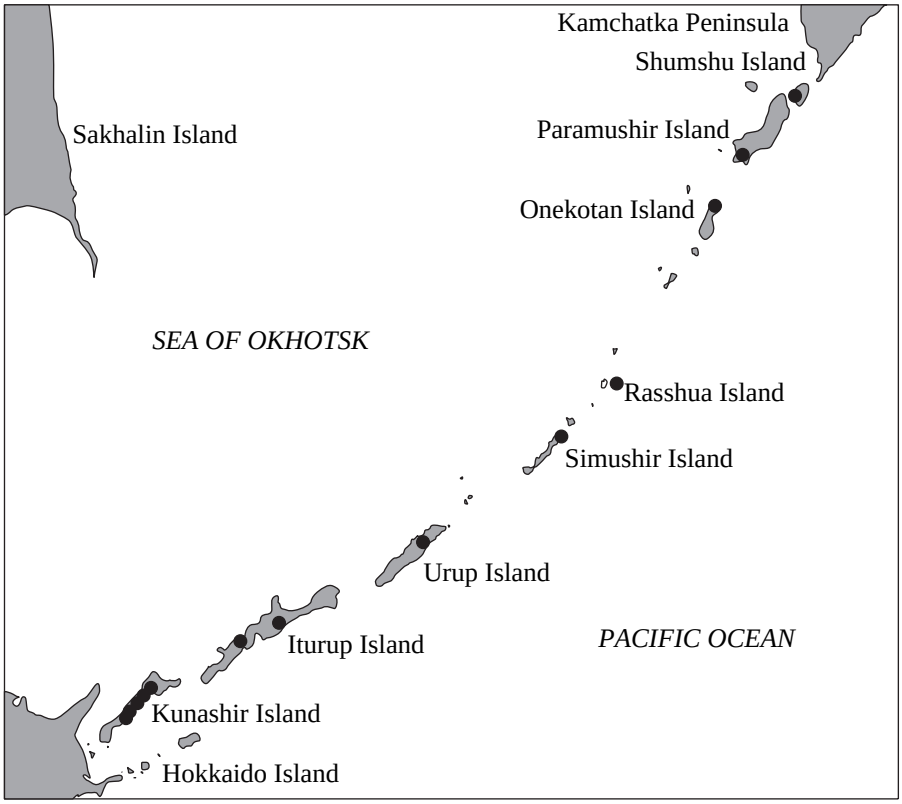

Fig. 1. Schematic map of sampling localities of *S. malma* populations: Shumshu Island (Bol'shaya Channel); Paramushir Island (Bol'shaya River); Onekotan Island (Nemo Brook); Rasshua Island (tributary of Nepristupnyi Brook); Simushir Island (nameless brook); Urup Island (Aleutka River); Iturup Island (Pioner River, coast of the Sea of Okhotsk; Kasatka River, oceanic coast); Kunashir Island (from south to north, Tyurina, Lesnaya, Petrovka, Prozrachnaya, and Ilyushina rivers).

expeditions conducted within the framework of the International Kuril Project. Fish from the Kunashir Island was sampled during the expedition of the Institute of Marine Biology, Russian Academy of Sciences, in 1997 (Fig. 1).

Shumshu Island. Fish was sampled at the mouth of the Bol'shaya Channel, which joins the Bol'shoe Lake with the Sea of Okhotsk.

Paramushir Island. Fish was sampled in the Bol'shaya River, which flows into the Vasil'eva Gulf at the southern extremity of the island.

*Onekotan Island. F*ish was sampled in the wider part (200 to 300 m from the mouth) of the Nemo Brook, which flows into the Nemo Bay in the northwest of the island.

Rasshua Island. Fish was sampled in the upper reaches of the Nepristupnyi Brook, which flows (forming a 20-m cascade) into the Nepristupnaya Bay on the oceanic side of the island [16].

Simushir Island. Fish was sampled at mouth of each of three small brooks located close to one another, which are tributaries of a larger nameless brook flowing into the Dushnaya Bay in the northeastern part of the island. The brook chars have the possibility of both exchange of individuals and outlet to the sea, thus forming a united gene pool; therefore, we studied these brook populations as a pooled sample.

Urup Island. Fish was sampled in the Aleutka River, which is located on the eastern side of the island and flows into the Aleutka Bay.

Iturup Island. Fish was sampled in rivers Pioner (Kuibyshevskii Gulf, Sea Of Okhotsk coast) and Kasatka (Kasatka Gulf, ocean coast).

Kunashir Island. Rivers Tyurin, Lesnaya, Petrovka, Prozrachnaya, and Ilyushin flow down the eastern slope of the island into the South-Kuril Strait. The populations from the two rivers listed first are isolated.

Thus, this study covered populations from all major Kuril islands. Except for the sample from the Rasshua population, which is isolated by a waterfall and consists only of the resident form, other samples may in addition to it contain an admixture of anadromous fish.

In total, 328 fish were examined (sample sizes are given in Table 2).

Electrophoresis of 19 enzymes was conducted in starch and polyacrylamide gels using several buffer systems [17–20]. The designations of loci follow the protein-coding gene nomenclature given in [21].

The allele frequencies for isolocus pairs *GPI-B1,2**, *IDDH-1,2**, *sMEP-B1,2**, and *mMEP-1,2** were esti-

Table 1. Enzymes and genetic loci encoding these enzymes in *S. malma* populations

Enzyme, E.C. no.	Loci	Tissue	Buffer system
Aspartate aminotransferase, 2.6.1.1	*sAAT-1,2**	M	CAME, pH 7.2
Alcohol dehydrogenase, 1.1.1.1	*ADH**	L	TBCL, pH 8.1/8.5
Glucose-6-phosphate isomerase, 5.3.1.9	*GPI-B1,2**	M	TBCL, pH 8.1/8.5
	*GPI-A**	M	″
1-iditol dehydrogenase, 1.1.1.14	*IDDH-1,2**	L	TBCL, pH 8.1/8.5
Isocitrate dehydrogenase, 1.1.1.42	*mIDHP-1**	M	CAME, pH 7.2
	*mIDHP-2**	M	″
	*sIDHP-3**	L	TBCL, pH 8.1/8.5
Acid phosphatase, 3.1.3.2	*ACP**	M	CAME, pH 7.2
Lactate dehydrogenase, 1.1.1.27	*LDH-A1**	M	TBCL, pH 8.1/8.5
	*LDH-A2**	M	″
	*LDH-B1**	M	″
	*LDH-B2**	M	″
Malate dehydrogenase, 1.1.1.37	*sMDH-B1,2**	M	TEB, pH 8.3
Malic enzyme, 1.1.1.40	*mMEP-1**	M	CAME, pH 7.2
	*mMEP-2**	M	″
	*sMEP-3**	M	″
Phosphomannose isomerase, 5.3.1.8	*MPI**	M	TEB, pH 8.3; TEB, pH 8.7
Peptidases, 3.4.-.-			
Phe–Pro	*PEPD-1**	M	CAME, pH 7.2
Leu–Tyr	*PEP-LT1**	M	CAME, pH 7.2; TEB, pH 8.7
Leu–Gly–Gly	*PEPB-1**	M	TBCL, pH 8.1/8.5
Superoxide dismutase, 1.15.1.1	*sSOD-2**	ML	TBCL, pH 8.1/8.5
Formaldehyde dehydrogenase	*FDHG**	M	TEB, pH 8.7
Phosphoglucomutase, 5.4.2.2	*PGM-1**	M	TBCL, pH 8.1/8.5
	*PGM-2**	M	″
6-phosphogluconate dehydrogenase, 1.1.1.44	*PGDH**	M	TEB, pH 8.3; TEB, pH 8.7
Esterase, 3.1.1.-	*EST-1**	L	TBCL, pH 8.1/8.5
	*EST-2**		″
	*EST-3**		″
	*EST-4**		″
	*EST-5**		″
Esterase D, 3.1.-.-	*EST-D**	M	TEB, pH 8.3

Note: M, muscles; L, liver. CAME, pH 7.2 [17]; TEB, pH 8.7 [18]; TEB, pH 8.3 [29]; TBCL, pH 8.1/8.5 [20].

mated by the maximum likelihood method proposed by Waples [22]. In view of small sample sizes, interpopulation allele heterogeneity based on allele frequencies was estimated by psudo-probability χ^2 test [23]. To estimate Nei's genetic distances [24] from allele frequencies, conduct UPGMA cluster analysis and multidimensional scaling, program packages BIOSYS-2 and SYSTAT were used. Interpopulation genetic diversity G_{ST} was estimated according to Nei [24].

A detailed analysis of genetic variation in the Kunashir populations was presented in our previous work [14]. Here, to examine interpopulation genetic differentiation we employed mean allele frequencies for five rivers from Kunashir and two rivers from Iturup (population of one of these rivers, Kasatka, was also studied by us earlier in the work cited above).

RESULTS AND DISCUSSION

The list of the studied enzymes, genetic loci controlling these enzymes, and buffer systems used is presented in Table 1.

Table 2. Frequencies of alleles of polymorphic loci in the Dolly Varden populations from rivers of the Kuril Islands

Loci	Populations (islands)								
	Shumshu	Paramushir	Onekotan	Rasshua	Simushir	Urup	Iturup[a]	Iturup[b]	Kunashir[c]
*ACP**									
**100*	0.136	0.000	0.310	0.354	0.100	0.000	0.026	0.087	0.102
**83*	0.864	1.000	0.690	0.646	0.900	1.000	0.974	0.913	0.898
N	11	13	29	24	0.40	16	38	55	138
*ADH**									
**-100*	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000
**-5*	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000
N	11	13	28	20	37	16	32	49	138
*EST-D**									
**100*	1.000	0.962	0.897	1.000	0.988	1.000	0.681	0.841	0.958
**120*	0.000	0.038	0.103	0.000	0.012	0.000	0.319	0.159	0.042
N	11	13	29	25	41	16	36	53	138
*FDHG**									
**100*	0.909	1.000	0.983	1.000	0.988	1.000	1.000	1.000	1.000
**128*	0.091	0.000	0.017	0.000	0.012	0.000	0.000	0.000	0.000
N	11	13	29	25	41	16	38	55	138
*GPI-B1**									
**100*	0.806	0.076	0.029	0.484	0.025	0.000	0.021	0.041	0.155
**70*	0.111	0.924	0.953	0.516	0.938	1.000	0.756	0.847	0.660
**35*	0.083	0.000	0.017	0.000	0.037	0.000	0.223	0.112	0.183
**5*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
N	9	13	29	25	40	16	38	55	138
*GPI-B2**									
**100*	0.806	0.655	0.747	0.536	0.625	0.750	0.703	0.644	0.865
**70*	0.111	0.345	0.253	0.464	0.375	0.250	0.297	0.356	0.132
**35*	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
**5*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003
N	9	13	29	25	40	16	38	55	138
*IDDH-1**									
**100*	1.000	1.000	0.964	1.000	0.912	1.000	0.953	0.977	0.916
**8*	0.000	0.000	0.036	0.000	0.088	0.000	0.047	0.023	0.084
**N*	11	13	28	20	34	16	32	49	100
*IDDH-2**									
**100*	1.000	1.000	0.964	1.000	0.912	1.000	0.953	0.977	1.000
**8*	0.000	0.000	0.036	0.000	0.088	0.000	0.047	0.023	0.000
N	11	13	28	20	34	16	32	49	100
*sIDHP-3**									
**100*	0.955	0.917	1.000	1.000	0.986	1.000	1.000	1.000	0.980
**120*	0.045	0.083	0.000	0.000	0.014	0.000	0.000	0.000	0.000
**85*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020
N	11	12	28	20	37	16	32	49	138
*sMDH-B1**									
**100*	0.909	1.000	1.000	1.000	0.887	0.906	0.960	0.930	0.794
**123*	0.091	0.000	0.000	0.000	0.000	0.094	0.040	0.070	0.206
**73*	0.000	0.000	0.000	0.000	0.113	0.000	0.000	0.000	0.000
N	11	13	29	25	40	16	38	55	138
*sMDH-B2**									
**100*	1.000	1.000	1.000	1.000	0.963	1.000	1.000	1.000	0.956
**123*	0.000	0.000	0.000	0.000	0.037	0.000	0.000	0.000	0.044
N	11	13	29	25	40	16	38	55	138

Table 2. (Contd.)

Loci	Populations (islands)								
	Shumshu	Paramushir	Onekotan	Rasshua	Simushir	Urup	Iturup[a]	Iturup[b]	Kunashir[c]
*mMEP-1**									
**100*	1.000	1.000	0.920	1.000	0.883	1.000	1.000	1.000	0.969
**120*	0.000	0.000	0.080	0.000	0.117	0.000	0.000	0.000	0.031
N	11	12	22	23	40	16	28	45	138
*mMEP-2**									
**100*	0.000	0.167	0.262	0.087	0.217	0.101	0.125	0.190	0.203
**120*	1.000	0.833	0.738	0.913	0.783	0.899	0.875	0.810	0.797
N	11	12	22	23	40	16	28	45	138
*sMEP-3**									
**100*	1.000	0.923	1.000	1.000	1.000	0.719	0.959	0.763	0.850
**88*	0.000	0.077	0.000	0.000	0.000	0.281	0.041	0.237	0.150
N	11	13	29	25	41	16	37	54	138
*PEPB-1**									
**100*	0.955	1.000	0.944	1.000	0.805	0.562	0.961	0.907	0.887
**122*	0.045	0.000	0.018	0.000	0.183	0.438	0.013	0.007	0.000
**65*	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000
**137*	0.000	0.000	0.019	0.000	0.012	0.000	0.026	0.086	0.113
N	11	13	27	25	41	16	38	55	138
*PEPD-1**									
**100 (70)*	0.500	0.692	0.759	0.660	0.866	0.656	0.934	0.879	0.686
**125 (100)*	0.500	0.077	0.069	0.340	0.000	0.031	0.000	0.044	0.300
**80*	0.000	0.231	0.172	0.000	0.134	0.313	0.066	0.077	0.014
N	11	13	29	25	41	16	38	55	138
*PEP-LT1**									
**100*	1.000	1.000	0.768	0.958	0.854	0.875	0.882	0.750	0.956
**170*	0.000	0.000	0.232	0.021	0.037	0.125	0.105	0.244	0.044
**130*	0.000	0.000	0.000	0.021	0.000	0.000	0.013	0.006	0.000
**60*	0.000	0.000	0.000	0.000	0.109	0.000	0.000	0.000	0.000
N	3	13	28	24	41	16	38	55	138
*PGDH**									
**100*	1.000	0.958	1.000	1.000	0.976	0.969	1.000	1.000	1.000
**105*	0.000	0.042	0.000	0.000	0.024	0.031	0.000	0.000	0.000
N	11	12	29	25	41	16	38	55	128
*PGM-2**									
**100*	0.955	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
**110*	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	11	13	29	25	41	16	38	55	138
*sSOD-2**									
**100*	0.091	0.769	0.293	1.000	0.976	1.000	0.987	0.994	0.580
**80*	0.909	0.231	0.673	0.000	0.012	0.000	0.013	0.006	0.420
**90*	0.000	0.000	0.034	0.000	0.012	0.000	0.000	0.000	0.000
N	11	13	29	25	41	16	38	55	138

Note: *N*, number of individuals studied.

[a] Allele frequencies for the population of the Pioner River.

[b] Averaged allele frequencies for the populations of the Pioner and Kasatka rivers.

[c] Averaged allele frequencies for the populations of the Tyurina, Lesnaya, Petrovka, Prozrachnaya, and Ilyushina rivers (see [14]).

In parentheses (see *PEPD-1**), the electrophoretic mobilities are given that we used previously [11].

Table 3. Genetic variability of 20 polymorphic loci in *S. malma* populations

Populations (islands)	Mean sample size per locus	Mean number of alleles per locus	Percent of polymorphic loci*	Mean heterozygosity, H_S
Shumshu	10.4 (0.4)	1.6 (0.2)	50.0	0.104 (0.031)
Paramushir	12.9 (0.1)	1.5 (0.1)	45.0	0.107 (0.035)
Onekotan	28.0 (0.5)	1.9 (0.2)	65.0	0.157 (0.039)
Rasshua	23.7 (0.4)	1.4 (0.1)	35.0	0.110 (0.043)
Simushir	39.7 (0.5)	2.0 (0.1)	85.0	0.144 (0.030)
Urup	16.0 (0.0)	1.5 (0.1)	40.0	0.118 (0.040)
Iturup	52.7 (0.8)	1.9 (0.2)	65.0	0.141 (0.034)
Kunashir	134.2 (2.6)	2.0 (0.2)	75.0	0.173 (0.038)

* At a frequency of major allele lower than 0.99.
In parentheses, standard error is given.

Of 36 loci studied, 20 were polymorphic ($P_{0.99}$) at least in one of the populations examined (Table 2); 11 loci were invariant in all studied samples: *sAAT-1*,*2**, *GPI-A**, *mIDHP-1**, and *-2**, *LDH-A1**, *-A2**, *-B1**, *–B2**, *MPI**, and *EST-1**. In the earlier studied populations of the southeastern Sakhalin Island, we detected variation of locus *PGM-1**, which conforms to the presence of null allele. In the populations studied in the present work, we did not detected any null-allele homozygotes for locus *PGM-1**, though observed some variation in the corresponding activity zone; i.e., we cannot exclude polymorphism by null allele, but the exact identification of genotypes was difficult. The variation by liver esterase loci *EST-2**, *-3**, *-4**, and *-5** could not be unambiguously interpreted in any sample. Therefore, we excluded loci *PGM-1**, *EST-2**, *-3**, *-4**, *-5** from further analysis. The remaining 20 polymorphic loci were used in the analysis of genetic differentiation of populations.

Some of the populations studied in this work (Table 2) differed from populations of the southern Dolly Varden examined earlier [14] by the allele composition of the loci. Thus, in addition to earlier detected alleles **137* and **65*, we found allele **122* of locus *PEPB-1** in the populations from the islands located north of the Kunashir Island. An allele with the same electrophoretic mobility was fixed in the Japanese char *S. leucomaenis* [15], which is a phylogenetically more ancient species than *S. malma* [25]. Therefore, allele **122* can be assumed more ancient. In the course of evolution, this allele was substituted by alleles **100*, **137*, and **65* in most *S. m. kraschenninikovi* populations but was preserved at an appreciable frequency in *S. m. kraschenninikovi* populations from the Kuril Islands. In *S. malma* populations from the Onekotan and Simushir islands, we found a rare allele *sSOD-2*90*, which is evidently similar to allele *sSOD-2*110* that was found earlier by Osinov and Pavlov [13] in a population from the southern Sakhalin Island. In addition, with noticeable frequency (0.109) the "slow" allele *PEP-LT1*60* (which is probably similar to allele *PEP-LT1*66* that was found in the *S. malma* population of the Hokkaido Island by Crane *et al*. [5]) was found only in the *S. malma* population of the Simushir Island. Furthermore, note the rare alleles that has not been earlier found in Asian *S. malma* populations: *PGM-2*110* (Shumshu Island) and *PGDH*105* (Paramushir, Urup, and Simushir islands). So far, because of a small size of the overwhelming majority of the studied samples throughout the species range, it is difficult to judge whether these alleles and allele *PEPB-1*122* are indeed specific of populations of the Kuril region.

In all studied samples for all polymorphic systems, we observed the conformity of the observed genotype ratio to the expected Hardy–Weinberg proportions; i.e., each sample represents a panmictic, genetically single population.

The proportion of polymorphic loci in the *S. malma* populations of the Kuril Islands varied from 35 to 85%; the mean expected heterozygosity (H_S) by 20 polymorphic loci exhibited a trend of a southward increase with the minimum in the most northern population of the Shumshu Island and the maximum in the most southern population of the Kunashir Island (0.104 and 0.173, respectively; see Table 3).

Interpopulation genetic heterogeneity was detected for 17 out of 20 polymorphic loci with maximum values of the χ^2 test for the following loci: *GPI-B1** (192.1, *d.f.* = 21, $P < 0.001$), *sMDH-B1** (119.2, *d.f.* = 14, $P < 0.001$), *PEPD-1** (145.2, *d.f.* = 14, $P < 0.001$), *PEP-LT1** (128.2, *d.f.* = 21, $P < 0.001$) and *sSOD-2** (233.0, *d.f.* = 14, $P < 0.001$). The differences between all studied population pairs were statistically significant. Using the pseudoprobability test for samples of a small size [23], we tested the significance of differences and revealed the maximum summed χ^2 values in the following population (island) pairs: Shumshu–Simushir (253.4; d.f. = 42; $P < 0.001$); Shumshu–Iturup (283.8; d.f. = 38; $P < 0.001$); Simushir–Kunashir (283.2; d.f. = 43; $P < 0.001$); and Iturup–Kunashir (226.4; d.f. = 39; $P < 0.001$). At the same time, we did not detect any cases of fixation of alternative alleles (which is typical of genetically close

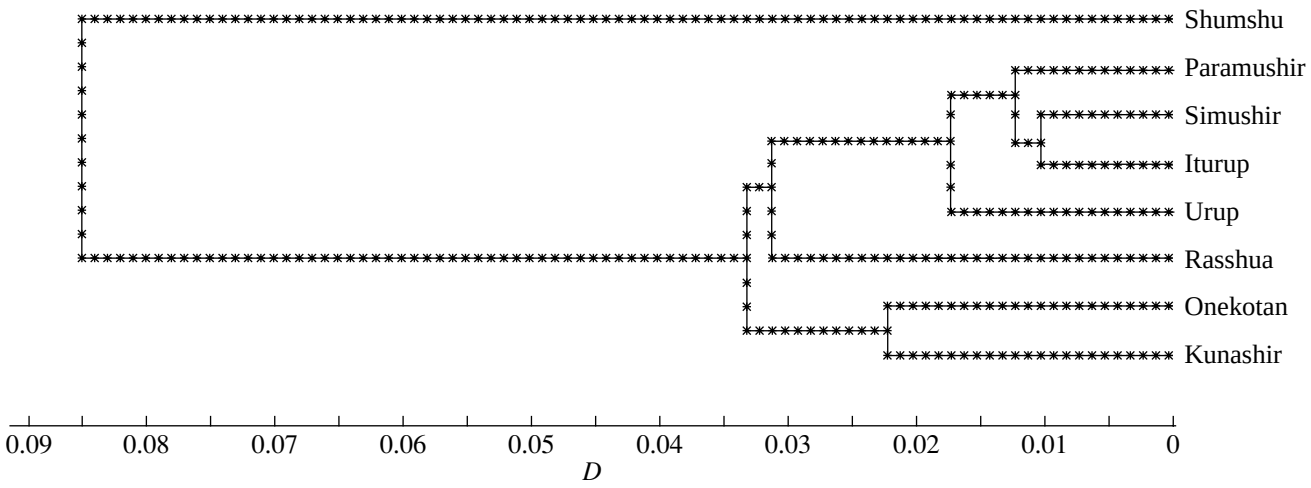

Fig. 2. Dendrogram of genetic similarity of *S. malma* from the Kuril Islands based on Nei's distances [24].

species belonging to the same genus) for any studied locus in the studied populations (Table 2). This indicates that the observed genetic differences lie within the limits of intraspecific differences and do not give grounds for subdividing the studied Dolly Varden from the Kuril Islands into different taxonomic groups.

The relative value of interpopulation (between the studied island populations) genetic diversity G_{ST} (calculated based on 20 common polymorphic loci) was 0.188, which is slightly greater than the interpopulation diversity among the studied populations of the Kunashir Island (0.170). Actually, the genetic diversity within the group of five populations of a single island, Kunashir Island, is comparable to the diversity among all studied populations of the Kuril Islands.

Cluster analysis of interpopulation genetic distances and their analysis by multidimensional scaling did not reveal any association between genetic similarity and geographical distances between populations (Figs. 2, 3). We detected a greater similarity within the Paramushir–Simushir–Urup–Iturup group of populations and a greater genetic divergence of the Kunashir, Onekotan, Rasshua, and especially Shumshu populations. The lack of association between genetic similarity and geographical position of islands in relation to one another can be partially explained by the fact that a small size of the used samples is not representative for the total *S. malma* populations of each studied island. In addition, as illustrated by the example of the Kunashir Island, the genetic diversity for the samples from rivers of a single island is comparable to the interpopulation genetic diversity for the samples representing different islands. Such heterogeneity of *S. malma* populations which we observed both within a single island and among islands, indicates that the interpopulation genetic exchange is very limited and even absent (in the case of physical isolation of populations). Indeed, although in many populations the presence of the anadromous *S. malma* form (which realizes this exchange) is not excluded, the potentialities of interpopulation migration of *S. malma* are limited by homing [26]. Hence, the genetic structure of many of these populations is mainly formed by genetic drift. In consequence of volcanic and tectonic processes at the islands, *S. malma* populations evidently experienced repeated reductions of population size, i.e., bottleneck effects and, in some cases, founder effects take place. All these factors provided the genetic heterogeneity of *S. malma* populations of the Kuril Islands.

Generally, the populations of the Kuril Islands are genetically different from the populations of the southern Sakhalin Island. A good illustration of this is a dendrogram (Fig. 4), in which the Sakhalin Island populations clustered separately from the populations of the Kuril Islands. The fact that an isolated population from the Rasshua Island fell into the cluster of populations from the Sakhalin Island is most probably caused by its random convergence with the Sakhalin group of populations at allele frequencies of some loci.

The *S. malma* sample from the channel of the Bol'shoe Lake (Shumshu Island) was maximally diverged from the populations of the Sakhalin Island and from other populations of the Kuril Islands. Its divergence is related to a high frequency of the common allele **100* of isoloci *GPI-B1,2** and to the fact that the frequency of the "slow" allele *sSOD-2*80* (earlier, this allele and the "fast" allele were designated as **100* and **115*, respectively [11]) in this population was the highest among all studied samples. At these loci, the differences of the sample from the Shumshu Island from other samples from the Kuril Islands were highly significant. As we already mentioned above, the presumed northern boundary of *S. malma kraschenninikovi* range lies between the northern Kuril Islands and the Kamchatka Peninsula [10]. In view of this, it is reasonable to suppose that the northern Dolly Varden can

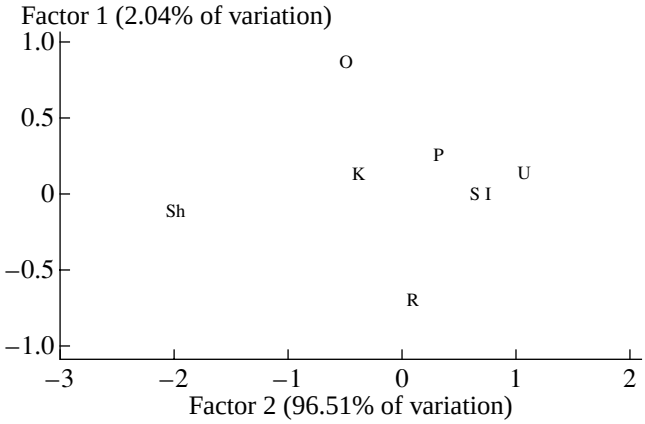

Fig. 3. Genetic differentiation of char populations inferred from multidimensional scaling. Designations: Sh, Shumshu Island; P, Paramushir Island; O, Onekotan Island; R, Rasshua Island; S, Simushir Island; U, Urup Island; I, Iturup Island; K, Kunashir Island.

occur in the most northern island (Shumshu Island) in addition to the southern Dolly Varden. We observed a bias in the allele frequencies of isoloci *GPI-B1*,*2** and locus *sSOD-2** in the direction of the values typical of populations of the northern Dolly Varden, in particular, *S. m. malma* populations of the Kamchatka Peninsula. *S. m. malma* populations are virtually monomorphic for allele *GPI-B1*,*2*100* and have a high frequency of allele *sSOD-2*80* [11, 13; our unpublished data].

The fact that the genetic distinctions making the sample from the Shumshu Island closer to the northern form of the Dolly Varden are concurrently observed for two discriminative loci indicates nonrandomness of these biases, i.e., the presence of *S. m. malma* genes in this sample. The concurrent presence of alleles *GPI-B1*,*2*70* and **35* which are typical of *S. malma krascheninnikovi*, suggest that we observe a mixture of the southern and northern forms of the Dolly Varden in this sample. The possibility of their mixing is also confirmed by finding the anadromous Dolly Varden that was identified by specific diagnostic features (the number of vertabrae, the number of scales in the lateral line, and the number of pyloric caeca) as the northern Dolly Varden in the same channel in the Shumshu Island [27]. We cannot exclude hybridization between two forms in this region, because we did not observed any heterozygote deficit for polymorphic loci (which occurs by virtue of Wahlund effect in a mechanic mixture of two genetically different groups), although a greater sample size may be necessary to reveal it. The boundary between the northern and southern forms does not seem so discrete as it was thought by Gritsenko *et al.* [10] (whose idea is that Dolly Varden from all islands of the Kuril Archipelago belongs to the southern form) but is not fuzzy. Apparently, the southern extremity of the Kamchatka Peninsula and the northern Kuril Islands represent a relatively narrow transition zone from one form to the other. This conclusion seems to be plausible, taking

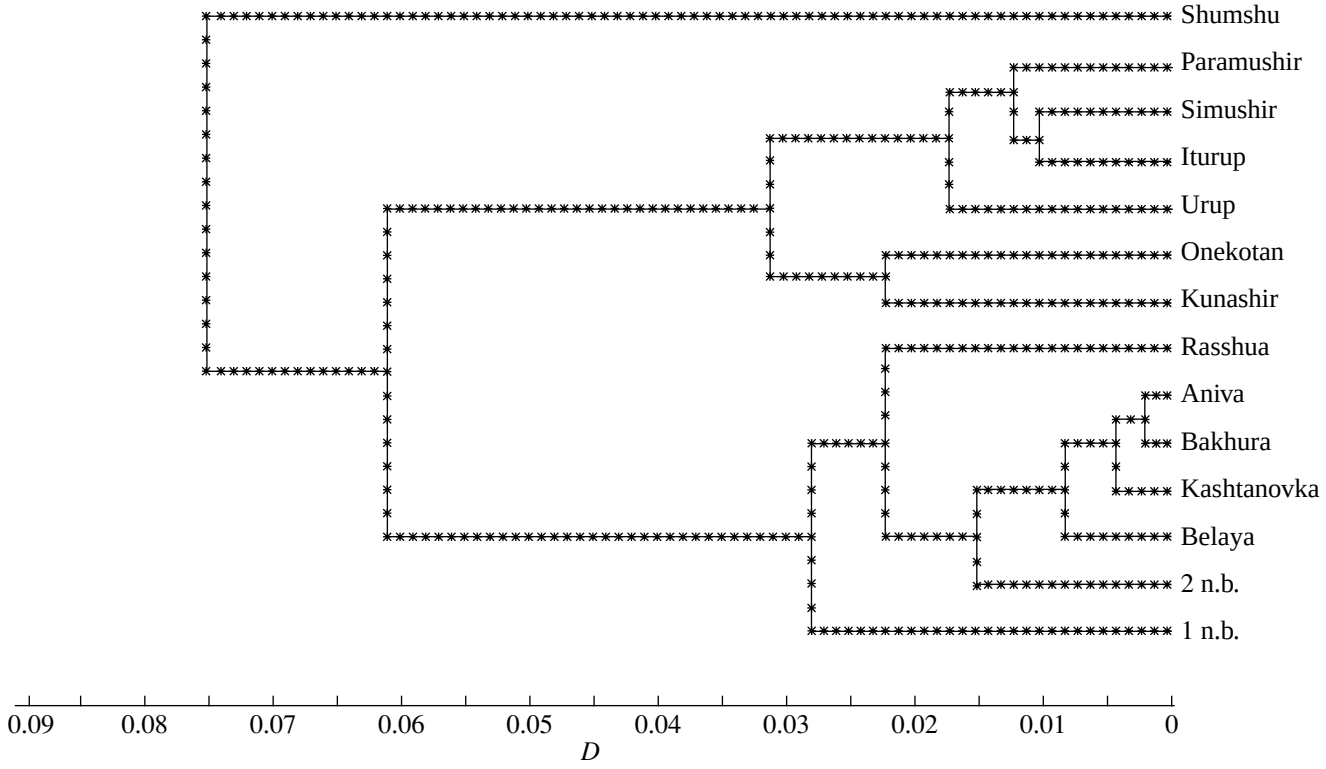

Fig. 4. Dendrogram of genetic similarity of *S. malma* from the Sakhalin Island and the Kuril Islands based on Nei's distances [24]. Designations: n.b. 1, first nameless brook; n.b. 2, second nameless brook.

into consideration the proximity of the Shumshu Island to the Kamchatka Peninsula and the lagoon origin of the Bol'shoe Lake [16].

It was repeatedly noted that the southern Dolly Varden exhibits a significantly higher level of genetic variation than the northern Dolly Varden [13, 14]. On the one hand, this difference can be explained by a significantly greater population size of the southern Dolly Varden (whose populations dwell under more favorable climatic conditions) and by repeated drops in population size of the northern Dolly Varden (bottleneck effect) because of severe overwintering conditions [14, 28]. On the other hand, the significant difference in genetic variation level of these two subspecies may be accounted for by historical reasons related to glaciation period. According to the opinion of Taranetz [29] and Gritsenko [30], the southern *S. malma* subspecies was formed during one of interglacial periods and was isolated from the northern subspecies for a long time, which was sufficient for the formation of significant karyological differences, on the territories that had not been exposed to glaciation [10]. By contrast, the northern subspecies underwent significant reductions in population size because of repeated glaciation throughout a considerable part of its range. Apparently, it was preserved in few refugia, from which it was spread into regions that became free from ice. This history could cause the low genetic variation of the northern Dolly Varden [31]. In view of this, it is interesting to note that the northern boundary of *S. m. kraschenninnikovi* range (which is related to the most northern islands of the Kuril Archipelago) virtually coincides with the boundary of the last Quaternary glaciation. The traces of this glaciation were found in the Paramushir Island, but they are absent southward of this island [16]. The view on a more ancient phylogenetic age of the southern Dolly Varden (compared to the northern Dolly Varden), based on allozyme data [31], is also supported by phylogeny (genealogy) analysis of mtDNA haplotypes of these subspecies [32].

Thus, the pattern of interpopulation genetic polymorphism of Dolly Varden from the Kuril Islands does not gives grounds to distinguish any distinct char species in this region other than *S. malma*, which is represented by the southern form with an admixture of the northern form in the most northern island (Shumshu Island). We did not found any case of genetic hiatus of populations (fixation of alternative alleles), which is a necessary condition for revision of the species status of populations.

The specific pattern of allozyme variation of *S. malma* populations from the Kuril Islands supports an assumption that the geographical boundary between the ranges of the northern and southern Dolly Varden subspecies lies approximately in latitude 50° north, which was earlier suggested on the basis of morphological differentiation [10].

ACKNOWLEDGMENTS

We thank Yu.N. Zhuravlev (Institute of Biology and Soil Sciences, Far Eastern Division of Russian Academy of Sciences) for kindly presenting the samples of the Dolly Varden from the northern and middle Kuril Islands and G.A. Rubtsova (Vavilov Institute of General Genetics, Russian Academy of Sciences) for technical help at some stages of work.

This work was supported by the Russian Foundation for Basic Research, grant no. 99-04-48591, and the Russian State Program "Leading Scientific Schools," grant no. 00-15-97914.

REFERENCES

1. Reshetnikov, Yu.S., Bogutskaya, N.G., Vasil'eva, E.D., *et al.*, A List of Freshwater Pisciformes and Fish of Russia, *Vopr. Ikhtiol.*, 1997, vol. 37, no. 6, pp. 723–771.
2. Cavender, T.M. and Kimura, S., Cytotaxonomy and Interrelationships of Pacific-Basin *Salvelinus*, *Physiol. Ecol. Jpn.*, 1989, vol. 1, pp. 49–68.
3. Phillips, R.B., Pleyte, K.A., and Ihssen, P.E., Patterns of Chromosomal Nuclear Organizer Region (NOR) Variation in Fishes of the Genus *Salvelinus*, *Copeia*, 1989, no. 1, pp. 47–53.
4. Gharrett, A.J., Goto, A., and Yamazaki, F., A Note on the Genetic Contrast of Sympatric Dolly Varden (*Salvelinus malma*) and Arctic Charr (*S. alpinus*) in the Karluk River System, Alaska, *Report of Oversea Work Supported by Grant-in-Aid for Overseas Sci. Serv. Ministr. Educat. Sci. Cult., Japan*, Yamazaki, F., Ed., 1991, pp. 37–48.
5. Crane, P.A., Seeb, L.W., and Seeb, J.E., Genetic Relationships among *Salvelinus* Species Inferred from Allozyme Data, *Can. J. Fish. Aquat. Sci.*, 1994, vol. 51, suppl. 1, pp. 182–197.
6. Omel'chenko, V.T., Politov, D.V., Salmenkova, E.A., *et al.*, Genetic Differentiation of Sympatric Chars of the Genus *Salvelinus* from the River Yama, *Genetika* (Moscow), 1996, vol. 32, no. 11, pp. 1562–1568.
7. Reist, J.D., Johnson, J.D., and Carmichael, T.J., Variation and Specific Identity of the Chars from Northwestern Arctic, Canada and Alaska, *Am. Fish. Soc. Symp.*, 1997, vol. 19, pp. 250–261.
8. Frolov, S.V., Frolova, V.N., and Molodichenko, A.V., The Karyotype of Dolly Varden *Salvelinus malma* and the Taxonomic Status of Northern and Southern Dolly Varden, *Biol. Morya*, 1997, no. 5, pp. 309–313.
9. Glubokovsky, M.K., *Evolyutsionnaya biologiya lososevykh ryb* (Evolutionary Biology of Salmonids), Moscow: Nauka, 1995.
10. Gritsenko, O.F., Savvaitova, K.A., Gruzdeva, M.A., and Kuzishchin, K.V., On the Taxonomic Status of Charr of the Genus *Salvelinus* from the Northern Kuril Islands, *Vopr. Ikhtiol.*, 1998, vol. 38, no. 2, pp. 189–198.
11. Omel'chenko, V.T., Salmenkova, E.A., Malinina, T.V., and Frolov, S.V., Genetic Differentiation of Sympatric Populations of Char of the Genus *Salvelinus* from the Lake Achchen (the Chukotka Peninsula), *Genetika* (Moscow), 1998, vol. 34, no. 3, pp. 399–405.
12. Omel'chenko, V.T., Nikiforov, S.N., and Malinina, T.V., Allozyme Variation and Genetic Differentiation of the

Dolly Varden (*Salvelinus malma* Walbaum) Population from Southeastern Sakhalin, *Genetika* (Moscow), 1998, vol. 34, no. 12, pp. 1655–1660.

13. Osinov, A.G. and Pavlov, S.D., Allozyme Variation and Genetic Divergence of Arctic Char and Dolly Varden Populations (*Salvelinus alpinus–S. malma* Complex), *Vopr. Ikhtiol.*, 1998, vol. 38, no. 1, pp. 47–61.
14. Salmenkova, E.A. and Omel'chenko, V.T., The Population Genetic Structure of Dolly Varden *Salvelinus malma* Walbaum from the Southeastern Sakhalin and the Southern Kuril Islands, *Genetika* (Moscow), 2000, vol. 36, no. 8, pp. 1100–1110.
15. Salmenkova, E.A., Omelchenko, V.T., Kolesnikov, A.A., and Malinina, T.V., Genetic Differentiation of Charrs in the Russian North and Far East, *J. Fish Biol.*, 2000, vol. 57, suppl. A, pp. 136–157.
16. Korsunskaya, G.V., *Kuril'skaya ostrovnaya duga* (The Kuril Island Arch), Moscow: Geograficheskaya Literatura, 1958.
17. Aebersold, P.B., Winans, G.A., Teel, D.J., *et al.*, Manual for Starch Gel Electrophoresis: A Method for the Detection of Genetic Variation, *NOAA Technical Report NMFS 61*, 1987.
18. Markert, C.L. and Faulhaber, I., Lactate Dehydrogenase Isozyme Patterns of Fish, *J. Exp. Zool.*, 1965, vol. 159, no. 2, pp. 319–332.
19. Peacock, A.C., Bunting, S.L., and Queen, K.G., Serum Protein Electrophoresis in Acrylamide Gel: Patterns from Normal Human Subjects, *Science*, 1965, vol. 147, pp. 1451–1452.
20. Ridgway, G.L., Shernburne, S.W., and Lewis, R.D., Polymorphism in the Serum Esterases of Atlantic Herring, *Trans. Am. Fish. Soc.*, 1970, vol. 99, pp. 147–151.
21. Shaklee, J.B., Allendorf, F.W., Morizot, D.C., and Whitt, G.S., Gene Nomenclature for Protein-Coding Loci in Fish, *Trans. Am. Fish. Soc.*, 1990, vol. 119, pp. 2–15.
22. Waples, R.S., Estimation of Allele Frequencies at Isoloci, *Genetics*, 1988, vol. 118, pp. 371–384.
23. Zaykin, D.V. and Pudovkin, A.I., Two Programs to Estimate Significance of χ^2 Values Using Pseudo-Probability Tests, *J. Hered.*, 1993, vol. 84, p. 152.
24. Nei, M., *Molecular Population Genetics and Evolution*, Amsterdam: North-Holland, 1975.
25. Behnke, R.J., Interpreting the Phylogeny of *Salvelinus*, *Physiol. Ecol. Jpn. Spec.*, 1989, vol. 1, pp. 35–48.
26. Chereshnev, I.A., *Biologicheskoe raznoobrazie presnovodnoi ikhtiofauny Severo-Vostoka Rossii* (Biological Diversity of Freshwater Fauna of Northeastern Russia), Glubokovsky, M.K., Ed., Vladivostok: Dal'nauka, 1996.
27. Shed'ko, S.V., An Overview of Freshwater Ichthyofauna, *Rastitel'nyi i zhivotnyi mir Kuril'skikh ostrovov (Materialy Mezhdunarodnogo Kuril'skogo proekta)* (Plants and Animals of the Kuril Islands: Proc. Int. Kuril Project), Storozhenko, S.Yu., Ed., Vladivostok: Dal'nauka, 2002, pp. 118–134.
28. Everett, R.J., Wilmot, R.L., and Krueger, C.C., Population Genetic Structure of Dolly Varden from Beaufort See Drainages of Northern Alaska and Canada, *Am. Fish. Soc. Symp.*, 1997, vol. 19, pp. 240–249.
29. Taranetz, A.Ya., Freshwater Fish of the Northwestern Basin of the Japanese Sea, *Tr. Zool. Inst. Akad. Nauk SSSR*, 1936, vol. 4, no. 2, pp. 483–537.
30. Gritsenko, O.F., Systematics and Origin of Sakhalin Chars of the Genus *Salvelinus*, *Tr. Vses. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr.*, 1975, no. 106, pp. 141–160.
31. Osinov, A.G., Evolutionary Relationships between the Major Taxa of the *Salvelinus alpinus–Salvelinus malma* Complex: The Results of Comparisons of the Allozyme Data Reported by Several Authors, *Vopr. Ikhtiol.*, 2001, vol. 41, no. 2, pp. 167–183.
32. Oleinik, A.G., Skurikhina, L.A., and Brykov, V.A., Mitochondrial DNA Divergence in Dolly Varden *Salvelinus malma* in the Asian Region of North Pacific, *Genetika* (Moscow) (in press).