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Genetic Differentiation of the Dolly Varden *Salvelinus malma* in Hokkaido, Japan

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Abstract

Samples of the Dolly Varden *Salvelinus malma* including one population of *S. malma miyabei* collected from 11 localities in the Hokkaido Island, Japan, were genetically characterized at 22 protein coding loci using starch-gel electrophoresis, in order to assess the genetic variability within and between populations, and to estimate genetic differentiation on both between the two subspecies, *S. malma malma* and *S. malma miyabei*, and among the populations of the former subspecies.

The genetic variability within each population were low (P=0-0.18 and H=0-0.045) while the genetic variability between them was high (Gst=0.48), suggesting that the population sizes of most *S. malma* populations in Hokkaido are small or have passed through a bottleneck effect in their recent history and that each population is isolated with no gene flow between them.

One population of *S. malma malma* from the Chihase River was found to differ markedly from other 9 populations of *S. malma malma* (D=0.058), showing as same genetic divergence as the population of *S. malma miyabei* from the 9 populations above (0.052). On the other hand, the high genetic differentiation of *S. malma miyabei* from the populations of *S. malma malma* supports the view that they should be given a taxonomic status as a subspecies.

Introduction

The Dolly Varden, *Salvelinus malma*, is widely distributed along the Pacific coast of North America from the Sacramento River to Seward Peninsula and along the Asian coast from the Yule River in Korea to the Anadyr River in Russia (McPhail and Lindsey, 1970). Because of their variable morphology and life-histories, the taxonomic status of the Dolly Varden has been confused for a great extent until recent years. For example, Barsukov (1960) concluded from morphological study on Chukotsk charr that there were no consistent differences between *S. malma* and *S. alpinus* and the former should be considered as a synonym of *S. alpinus*. Savvaitova (1961, 1976) also treated *S. malma* as a synonym of *S. alpinus*. On the other hand, Behnke (1972) pointed out that the Dolly Varden in North America deserved full species status but nomenclatorial problems could arise due to the fact that the type locality for the name *S. malma* is Kamchatka. Morrow (1980) demonstrated the close morphological similarities between the charr of northwestern Alaska and the most common charr of northeastern Siberia including Kamchatka, and concluded that the northwestern Alaskan charr should be classified not as *S. alpinus* but as *S. malma*. Since then, most authors have followed Morrow's view.

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In the Japanese Archipelago, the distribution of *S. malma* is restricted to the Hokkaido Island. On Hokkaido, this species is classified into two subspecies, *S. malma malma* and *S. malma miyabei*, which are distinguishable by morphological characteristics such as longer pectoral fins and larger number of gill-rakers for *S. malma miyabei* (Miyadi et al., 1963). The former subspecies, *S. malma malma*, is mainly distributed in the mountainous regions of Hokkaido and is composed of the river-resident form in all parts of its distribution except in some rivers in Shiretoko Peninsula on the eastern end of the island, where a few fish of the anadromous form were also found (Hikita, 1962; Maekawa, 1973; Komiyama et al., 1982). The latter subspecies, *S. malma miyabei*, is distributed only in Lake Shikaribetsu and its inlet streams on the Taisetsu Mountain of central Hokkaido, and consists of lake-run and river-resident forms (Kubo, 1976; Maekawa, 1977a, 1985).

Although there are some studies on the morphological variability and different life history of this species (Kubo, 1976; Maekawa, 1977a, b, 1985), few studies have been made of the genetic differentiation between and within each two subspecies. Recently, Cavender and Kimura (1989) demonstrated through a cytogenetic study that *S. malma miyabei* differed from the Hokkaido *S. malma malma* in the type and location of nucleolar organizer regions on chromosomes. The main objectives of the present study are to assess the genetic variability within and between populations of *S. malma* in Hokkaido and to estimate genetic differentiation between the two subspecies and among the populations of *S. malma malma*.

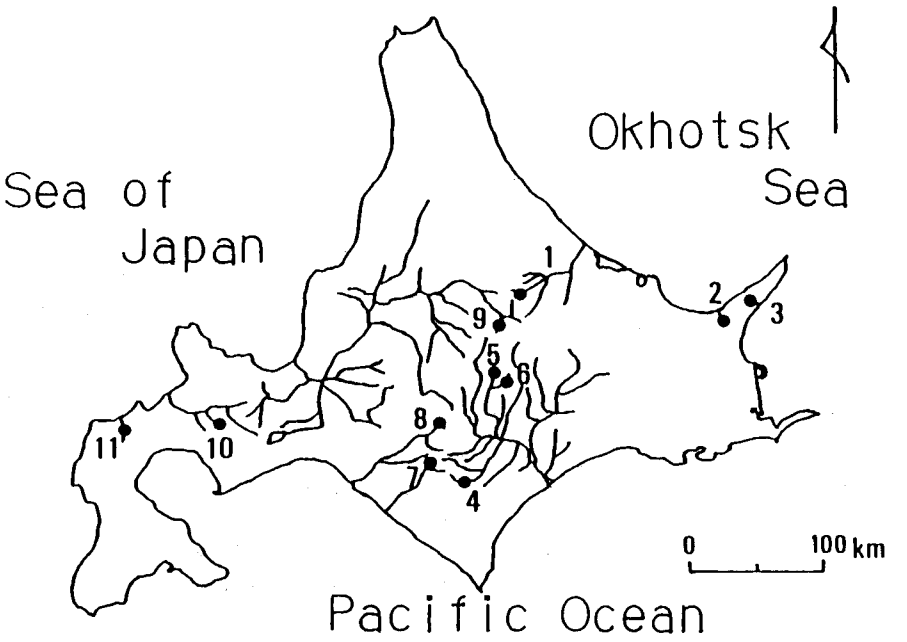

Fig. 1. Sites of collections for *Salvelinus malma* from Hokkaido.
1. Yubetsu River, 2. Ochikabake River, 3. Rausu River, 4. Tottabetsu River, 5. Euyambetsu River, 6. Yambetsu River, 7. Niikappu River, 8. Saru River, 9. Ishikari River, 10. Makkari River, 11. Chihase River.

Materials and Methods

Collection of specimens

Samples of populations of *S. malma malma* and *S. malma miyabei* were collected from 11 localities in the Hokkaido Island, Japan (Fig. 1). The sample collection sites, collection designations and sample sizes are as follows: 1) Yubetsu River (Yu); n=52, 2) Ochikabake R. (Oc), n=40; 3) Rausu R. (Ra), n=41; 4) Tottabetsu R. (To), n=44; 5) Euyambetsu R. (Eu), n=42; 6) Yambetsu R. (Ya), n=50 (To, Eu and Ya are the tributary of Tokachi River system); 7) Niikappu R. (Ni), n=52; 8) Saru R. (Sa), n=40; 9) Ishikari R. (Is), n=52; 10) Makkari R. (Ma), n=33; and 11) Chihase R. (Ch), n=50. Among them, only the samples from Yambetsu River were identified as *S. malma miyabei* and all the other samples were *S. malma malma*. Specimens were immediately frozen on dry ice and were stored at the laboratory in a freezer at −20° or −80°C until electrophoretic analysis.

Analysis of isozymes by starch gel electrophoresis

Horizontal starch gel electrophoresis of extracts of liver and skeletal muscle samples performed in C-APM (Clayton and Tretiak, 1972), CT (Clayton and Gee,

Table 1. Enzyme coding loci, Enzyme Commission (EC) number and locus designations, and the tissues and buffers in which they were resolved.

Enzyme	EC No.	Locus	Tissue	Buffer
Alcohol dehydrogenase	1.1.1.1	*Adh*	Liver	Ridgway
Aspartate aminotransferase	2.6.1.1	*Aat*	Muscle	CT
α-Glycerophosphate dehydrogenase	1.1.1.8	*Agp-1*	Muscle	C-APM
		Agp-2	Muscle	C-APM
Esterase	3.1.1.1	*Est*	Liver	Ridgway
Isocitrate dehydrogenase	1.1.1.42	*Idh-1*	Muscle	CT
		Idh-2	Muscle	CT
Lactate dehydrogenase	1.1.1.27	*Ldh-1*	Muscle	Ridgway
		Ldh-2	Muscle	Ridgway
		Ldh-3	Liver	Ridgway
		Ldh-4	Muscle	Ridgway
Malate dehydrogenase	1.1.1.37	*Mdh-1*	Liver, Muscle	C-APM
		Mdh-2	Liver, Muscle	C-APM
		Mdh-3, 4	Muscle	CT
Malic enzyme	1.1.1.40	*Me-1*	Muscle	C-APM
		Me-2	Muscle	C-APM
		Me-3	Muscle	C-APM
Phosphoglucomutase	5.4.2.2	*Pgm*	Muscle	Ridgway
6-Phosphogluconate dehydrogenase	1.1.1.44	*Pgd*	Muscle	CT
Sorbitol dehydrogenase	1.1.1.14	*Sdh-1*	Liver	Ridgway
		Sdh-2	Liver	Ridgway
Superoxide dismutase	1.15.1.1	*Sod*	Liver	Ridgway

1969) and Ridgway (Ridgway et al., 1970) buffers resolved twelve enzymes encoded by 22 loci (Table 1): alcohol dehydrogenase (ADH, EC 1.1.1.1), aspartate aminotransferase (AAT, EC 2.6.1.1), α-glycerophosphate dehydrogenase (AGP, EC 1.1.1.8), esterase (EST, EC 3.1.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), lactate dehydrogenase (LDH, EC 1.1.1.27), malate dehydrogenase (MDH, EC 1.1.1.37), malic enzyme (ME, EC 1.1.1.40), phosphoglucomutase (PGM, EC 5.4.2.2), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), sorbitol dehydrogenase (SDH, EC 1.1.1.14) and superoxide dismutase (SOD, EC 1.15.1.1). Staining procedures followed Shaw and Prasad (1970) with minor modifications.

The proportion of polymorphic loci (P), an indicator of genetic variability within population, was calculated for each collection. The mean of heterozygosities over all 22 loci estimated the average heterozygosity (H) for each population (Nei, 1978). Genetic distance (D; Nei, 1972) was calculated for each pairwise combination of populations. A dendrogram was constructed from the genetic distance matrix following the UPGMA procedure (Sneath and Sokal, 1973) to estimate the genetic relationship among the populations.

Results

Of 22 loci examined, six loci (*Adh*, *Mdh-1*, *Mdh-3, 4*, *Me-3*, *Pgm* and *Sdh-1*) were variable and five loci (excluding *Adh*) were polymorphic (*i.e.* the frequency of the common allele was 0.95 or less for at least one population) (Table 2). The other 16

Table 2. Allele frequencies observed at 6 variable loci, proportion of polymorphic loci (P) and average heterozygosity (H) in 11 population samples of Dolly Varden collected from Hokkaido. The figures in parentheses indicate the number of fish examined.

Locus	Alleles	Population samples										
		Yu (52)	Oc (40)	Ra (41)	To (44)	Eu (42)	Ya (50)	Ni (52)	Sa (40)	Is (52)	Ma (33)	Ch (50)
Adh	−100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.980
	−165	—	—	—	—	—	—	—	—	—	—	0.020
Mdh-1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	—
	24	—	—	—	—	—	—	—	—	—	—	1.000
Mdh-3, 4	128	0.010	—	0.085	—	0.220	0.075	—	—	—	—	—
	100	0.990	1.000	0.915	1.000	0.780	0.925	1.000	1.000	1.000	1.000	1.000
Me-3	100	0.962	0.887	0.988	0.784	1.000	0.060	1.000	0.375	0.106	0.455	1.000
	66	0.038	0.113	0.012	0.216	—	0.940	—	0.625	0.894	0.545	—
Pgm	123	—	—	—	—	—	—	—	—	—	0.015	—
	100	1.000	1.000	1.000	1.000	1.000	0.820	1.000	1.000	1.000	0.985	1.000
	84	—	—	—	—	—	0.180	—	—	—	—	—
Sdh-1	100	0.971	1.000	1.000	0.466	0.976	0.200	1.000	0.662	0.923	1.000	1.000
	2	0.029	—	—	0.534	0.024	0.800	—	0.338	0.077	—	—
P		0.00	0.05	0.05	0.09	0.05	0.18	0.00	0.09	0.09	0.05	0.00
H		0.008	0.009	0.015	0.038	0.031	0.045	0.000	0.042	0.015	0.024	0.002

loci were fixed for the same allele in all populations. In chi-square contingency tests, observed genotype frequencies of all polymorphic loci in each population conformed with Hardy-Weinberg expectations.

Genetic variability within populations

The proportion of polymorphic loci (P) in 11 population samples ranged from 0 to 0.18 (0.06±0.05 for mean±SD) and average heterozygosity (H) ranged from 0 to 0.045 (0.021±0.016) (Table 2).

Genetic differentiation between populations

The GST value, an indicator of genetic differentiation between populations, over all 11 populations was 0.48. The genetic distance between population pairs ranged from 0.000 to 0.126 (Table 3). The genetic relationships among 11 populations are shown in Figure 2, using the UPGMA clustering algorithm. The 5 populations from the Yubetsu River, Niikappu River, Rausu River, Ochikabake River and Euyambetsu River were almost homogenous (D=0.000-0.003) and the Tottabetsu River population differed somewhat from these 5 populations (D=0.013-0.017). The 3 populations from Ishikari River, Makkari River and Saru River were similar (D=0.006-0.007). The mean genetic distance between the former 6 populations and the latter 3 populations was 0.023. These 9 populations composed of *S. malma malma* (we call these 9 populations as "main group" hereafter) were markedly different from the Yambetsu River population which was composed of *S. malma miyabei* (D=0.030-0.075). At two loci (*Pgm* and *Sdh-1*), obvious differences in allele frequency

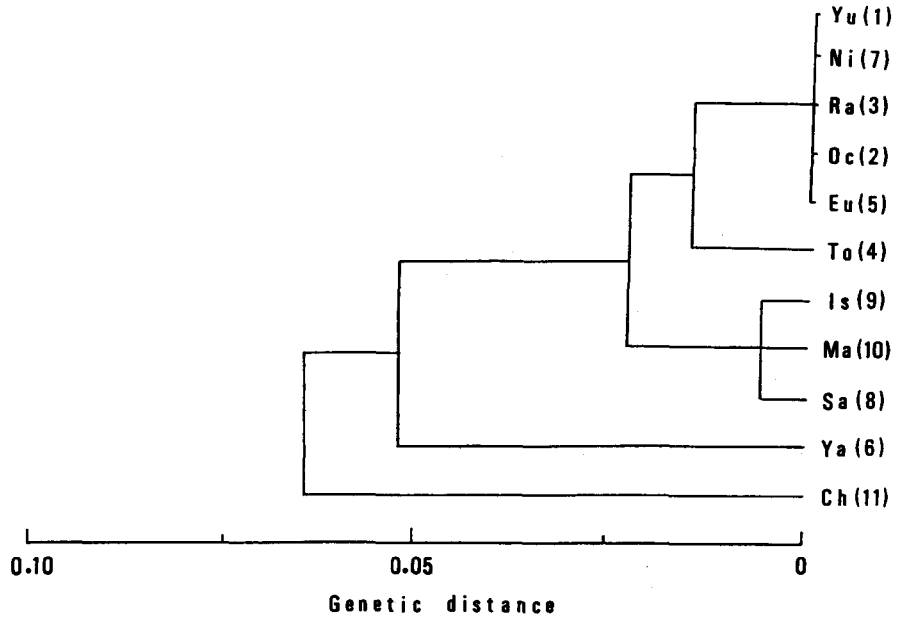

Fig. 2. UPGMA phenogram derived from Nei's genetic distances for *Salvelinus malma* populations in Hokkaido, based on 22 loci.

Table 3. Estimates of Nei's genetic identities (above diagonal) and genetic distances (below diagonal) for pairs of population samples of Dolly Varden, based on 22 loci.

Populations	Yu	Oc	Ra	To	Eu	Ya	Ni	Sa	Is	Ma	Ch
Yu	—	1.000	1.000	0.987	0.998	0.933	1.000	0.980	0.966	0.988	0.954
Oc	0.000	—	0.999	0.986	0.997	0.937	0.999	0.983	0.972	0.991	0.954
Ra	0.000	0.001	—	0.985	0.999	0.929	1.000	0.977	0.964	0.987	0.954
To	0.013	0.014	0.016	—	0.983	0.970	0.985	0.990	0.969	0.982	0.938
Eu	0.002	0.003	0.001	0.017	—	0.928	0.998	0.975	0.961	0.984	0.952
Ya	0.069	0.065	0.074	0.030	0.075	—	0.928	0.983	0.974	0.961	0.881
Ni	0.000	0.001	0.000	0.015	0.002	0.075	—	0.977	0.963	0.986	0.954
Sa	0.021	0.018	0.023	0.010	0.025	0.017	0.023	—	0.994	0.994	0.930
Is	0.034	0.029	0.037	0.032	0.040	0.027	0.038	0.007	—	0.994	0.917
Ma	0.012	0.009	0.014	0.019	0.016	0.040	0.014	0.006	0.006	—	0.940
Ch	0.047	0.047	0.047	0.064	0.049	0.126	0.047	0.072	0.086	0.062	—

were observed between them and the mean genetic distance between them was 0.052. Although the Chihase River population was composed of *S. malma malma*, it differed conspicuously from the other 10 populations (D=0.047-0.126), showing the greatest divergence from the Yambetsu River population, *S. malma miyabei*. At *Mdh-1*, the Chihase River population was fixed for a different allele from that of the other 10 populations. The mean genetic distance between them was 0.065.

Discussion

The genetic variability within populations for the 11 *S. malma* collections from Hokkaido were very low (P=0-0.18 and H=0-0.045). Such low variability may be due to their small population size or bottlenecks in their recent history and their geographic isolation in rivers or lakes. This seems to be consistent with the following results: among them, the populations living in relatively larger rivers such as the Tottabetsu River, Euyambetsu River and Saru River populations and the Yambetsu River population which lives in a lake as its habitat revealed a somewhat more variability than those observed for populations living in small rivers or in a limited small tributary (Yubetsu River, Ochikabake River, Niikappu River and Chihase River).

On the other hand, the genetic differentiation between populations of *S. malma* in Hokkaido was very high (G_{ST}=0.48) and was as same or higher than those of landlocked Arctic charr (*S. alpinus*) in Sweden (Anderson et al., 1983), North America (Kornfield et al., 1981) and Ireland (Ferguson, 1981) (G_{ST}=0.24, 0.52 and 0.43 respectively). This suggests that most populations of *S. malma* in Hokkaido are isolated and the gene flow between them is scarce.

Nine populations of the main group clustered at genetic distances less than 0.025 and the mean of genetic distances between them was 0.014. With the exception of the Saru River population, these populations subclustered into two local groups: one group from the rivers flowing into the Okhotsk Sea and Pacific Ocean (Yubetsu

River, Ochikabake River, Rausu River, Tottabetsu River, Euyambetsu River and Niikappu River) and the other group from the rivers along the Sea of Japan (Ishikari River and Makkari River). The genetic structure of the population from Saru River, though it flows into the Pacific Ocean, was similar to those of the Ishikari River and Makkari River populations. This suggests that the Saru River population might have originated from the Ishikari River population by headwater capture between the two rivers following the recent glacial age, considering the close location of the upper reaches between them, less than 10 km apart. However, the genetic similarity shown above might have been caused by a random genetic drift at *Me-3*. In order to elucidate the more detailed genetic structure, further analysis are needed with more polymorphic loci.

In contrast, the Chihase River population of *S. malma malma* differed markedly from the main group which belongs to the same subspecies (D=0.047-0.086). The mean genetic distance between them was 0.058, showing as same divergence as the Yambetsu River population (*S. malma miyabei*) from the main group (D=0.052). The large genetic divergence of the Chihase River population may be attributed to a strong bottleneck effect, small population size with high random genetic drift or natural selection. Besides the genetic character, the peculiarity of the Chihase River population was also found on its morphological characters such as markedly dwarf appearance (larger head length and eye diameter), smaller body size in comparison with the other populations in Hokkaido at the same age and reduction or lack of red spots on its body (Mitsuboshi, unpublished data). In the Chihase River which corresponds to the southern limit of the Dolly Varden distribution in Hokkaido, the charr are isolated above impassable waterfalls in the Hiyamizu-sawa stream (a small tributary of Chihase River) The population size is small, probably less than 1,000 adult individuals (Goto, pers. obs.). The low average heterozygosity (H=0.002) in the Chihase River population is also consistent with its narrow distribution and relatively small population size. Further study on morphological and reproductive characters and feeding habits of this population and further analysis on genetic structure of some populations around the southern limit of the Dolly Varden distribution in Hokkaido should be performed to bring the characteristics of this peculiar population into relief.

The population of *S. malma miyabei* from the Yambetsu River also differed greatly from the Chihase River population (D=0.126) and the main group (D=0.030-0.075). *S. malma miyabei* is distributed only in Lake Shikaribetsu and its inlet streams and has distinctive ecomorphological characteristics as compared to those of *S. malma malma* populations in Hokkaido. *S. malma miyabei* has the most numerous gill-rakers which range in number from 23 to 29 with a mode of 26 (Maekawa, 1977b). They utilize the lake instead of the sea as their habitat and feed mainly on zooplankton (Hada and Tomita, 1949), while the anadromous Dolly Varden are piscivorous during their ocean life (Ross, 1959) and the river-residents are insectivorous throughout their life (Ishigaki, 1984). In addition, an electrophoretic analysis revealed that the hemoglobin pattern of *S. malma miyabei* was distinctly different from that of the river-resident Dolly Varden from Hokkaido (Yoshiyasu, 1973). From these facts, Maekawa (1977a, b, 1985) hypothesized that such numerous gill-rakers in *S. malma miyabei* might be an adaptation to plankton feeding after the fish became landlocked in Lake Shikaribetsu following the recent

glacial age and he proposed that they should be regarded as a subspecies of *S. malma* and not as a distinct species *S. miyabei* (Okada and Nakamura, 1948; Oshima, 1961) or stock of typical anadromous *S. malma* (Kubo, 1967).

The relatively high genetic variability ($P=0.18$ and $H=0.045$) suggest an alternative explanation. It is possible that the large genetic divergence of *S. malma miyabei* from the river-resident populations of Hokkaido which were observed in this study may not be due to stochastic processes associated with limited population size in the lake; but, rather, may have occurred as the result of long geographic isolation from the local river resident populations and concomitant natural selection. Regardless, the distinctiveness of allozymic profile as well as that of ecomorphology in *S. malma miyabei* supports the view that they should be given a taxonomic status as a subspecies (Miyadi et al., 1963; Maekawa, 1985).

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