ミヤベイワナ(Salvelinus malma miyabei)とオショロコマ(Salvelinus malma malma)の遺伝的分化

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Genetic Differentiations of the Miyabe Charr, *Salvelinus malma miyabei* and Dolly Varden, *Salvelinus malma malma*

By

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Summary : It is generally considered that *Salvelinus malma miyabei* which inhabits Sikaribetsu Lake in Hokkaido is a subspecies of *S. m. malma*. To clarify the differentiation level between *Salvelinus m. miyabei* and *S. m. malma*, we investigated genetic differences and genetic differentiation using protein polymorphism and isozyme mutation among 3 groups (*S. m. miyabei* population, *S. m. malma* of the Tokachi River water system population which is close to Shikaribetsu Lake and *S. m. malma* of other region populations).

Of 11 loci examined, the S band and a′ band were detected in the *Hb-II* locus and *MDH* locus respectively for the Shikaribetsu Lake population, these bands are new varieties that were not previously detected in *S. m. malma*. Therefore, two newly identified loci that could distinguish *S. m. miyabei* and *S. m. malma* clearly are reported.

As a result of constructing a dendrogram using genetic distances (D) calculated from the allele frequency of 11 kind loci, the Tokachi River water system population (*S. m. malma*) and the other region populations (*S. m. malma*) were connected first in D=0.017, secondly these 2 populations and Shikaribetsu Lake population were connected in D=0.161. Therefore, *S. m. miyabei* was clearly classified as a subspecies of *S. m. malma*.

Key Words : Japanese charr, protein polymorphism, isozyme mutation, genetic differentiation, UPGMA method

Salvelinus malma miyabei is a Japanese charr that became landlocked in Lake Shikaribetsu and its inlet streams Yambetsu River etc. in the Mt. Taisetsuzan national park. It is generally considered that *S. m. miyabei* is a subspecies of *S. m. malma* (MIYADI *et al.*, 1963[1] ; MAEKAWA, 1985[2]). A difference between *S. m. miyabei* and *S. m. malma* was detected in morphological research by MAEKAWA (1977)[3] and biochemical research such as comparison of hemoglobin (Hb) patterns using starch gel electrophoresis by YOSHIYASU (1973)[4] and isozyme analysis of 22 loci by MITSUBOSHI (1992)[5]. However, the genetic locus showing a peculiar *S. m. miyabei* mutation involves only one locus of the Pgm type of the blood cell isozyme, and the effective genetic locus for clearly classifying two subspecies of *S. m. miyabei* and *S. m. malma* is limited. Furthermore, there are few features in the appearance that clearly distinguishing the two fish species. To express features on the taxonomy of *S. m. miyabei*, it is necessary to retrieve genetic marker loci using biochemical mutation. To clarify the differentiation level of *S. m. miyabei*, we examined genetic differences between *S. m. miyabei* and *S. m. malma* using polymorphic proteins[6] that we established, and investigated isozyme mutation.

Materials and Methods

Samples for the experiment were 30 mature *S. m. miyabei* fish (Total length ; over 200 mm) which Hokkaido Shikaoi town office transferred in 2000 and 249 mature *S. m. malma* fish (Total length ; over 180

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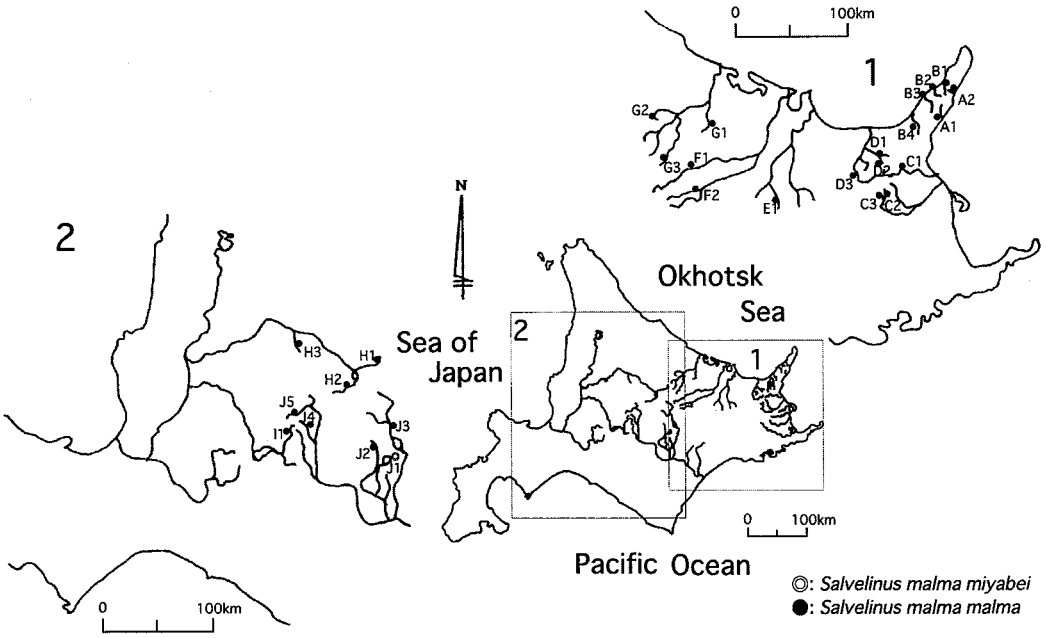


Fig. 1 Map showing sampling stations for *Salvelinus malma* from Hokkaido
J1 position in No. 2 shows Lake Shikaribetsu, J2-J5 position in No. 2 show Tokachi River water system, and A1-I1 in No. 1 and No. 2 show other River water system as shown in Table 1.

Table 1 Location of the area or water system where *Salvelinus malma* from Hokkaido were collected

Map code*	Location for sampling	Area or watar system
A1	Sakimui River	Rausu
A2	Rausu R.	Rausu
B1	Pereke R.	Utoro
B2	Opekepu R.	Utoro
B3	Kinzan R.	Utoro
B4	Shimatokari R.	Utoro
C1	Churui R.	Shibetsu
C2	Paushibetsu R.	Shibetsu
C3	Paushibetsu fork	Shibetsu
D1	Ikushina R.	Shari R.
D2	Akino R.	Shari R.
D3	Satturu R.	Shari R.
E1	Senuntubetsu R.	Abashiri R.
F1	Keshomappu R.	Tokoro R.
F2	Kamihokatokoro R.	Tokoro R.
G1	Muri R.	Yubetsu R.
G2	Yubetsu R.	Yubetsu R.
G3	Yubetsu fork	Yubetsu R.
H1	Rubeshibe R.	Ishikari R.
H2	Yambetappu R.	Ishikari R.
H3	Kuwaunnai R.	Ishikari R.
I1	Ikutora R.	Sorachi R.
J1	Lake Shikaribetsu	Tokachi R.
J2	Ishikaribetsu R.	Tokachi R.
J3	Otofuke R.	Tokachi R.
J4	Sahoro R.	Tokachi R.
J5	Kamuisankenai R.	Tokachi R.

*Refer to Fig. 1.

Table 2 Allele frequencies at the 11 loci, proportion of polymorphic loci (*P poly*) and average heterozygosity ($\overline{H}$) in 3 populations of *Salvelinus malma* cllected from Hokkaido

Locus	Alleles	Lake Shikaribetsu	Tokachi River water system	Other River water system
		S. m. miyabei	*S. m. malma*	*S. m. malma*
Tf	*D*	0	0.083	0.011
	F	0.933	0.576	0.807
	H	0.067	0.091	0.168
	J	0	0	0
	L	0	0	0.014
	-	0	0.250	0
		(30)	(42)	(182)
Es- I	*D1*	0	0	0
	D2	0	0	0
	D3	0	0	0
	-	1.000	1.000	1.000
		(30)	(42)	(182)
Es- II	*A*	0	0.036	0.160
	B	0	0.061	0.056
	-	1.000	0.903	0.784
		(30)	(42)	(182)
Es- III (i)	*D*	0	0.048	0.056
	E	0	0.024	0.022
	F	0	0.199	0.126
	-	1.000	0.729	0.796
		(30)	(42)	(182)
Es- III (ii)	*I*	0.417	0.518	0.416
	S	0.462	0.181	0.275
	T	0.083	0.012	0.005
	-	0.038	0.289	0.304
		(30)	(42)	(182)
Hb- I	*A*	0	0.024	0.116
	B	1.000	0.976	0.884
Hb- II	*F*	0.200	1.000	1.000
	S	0.800	0	0
		(30)	(42)	(207)
Cell X	*A*	0.017	0.012	0.084
	B	0.983	0.988	0.916
		(30)	(42)	(202)
Mu	*A*	1.000	1.000	0.995
	B	0	0	0.005
		(30)	(42)	(202)
MDH	*a*	0	0.022	0.030
	a'	0.058	0	0
	b	0.942	0.978	0.970
		(30)	(23)	(74)
SDH	*a*	0.554	0.643	0.816
	b	0.446	0.357	0.184
		(28)	(21)	(49)
Ppoly		0.455	0.455	0.636
$\overline{H}$		0.153	0.217	0.219

( ) :Number of individuals.

mm) collected from 26 rivers in 1997-2000 by fishing. And as a comparison fish, we used *S. leucomaenis* (whitespoted charr).

Each individual fish was analyzed after dividing *S. m. miyabei* into 3 groups based on the collection site : Shikaribetsu Lake (J1), Tokachi River water system (J 2~J5) which is near Shikaribetsu Lake and the other remote location (A1~I1) which is further than Tokachi River water system (Table 1, Fig. 1). Serum, hemoglobin (Hb), blood cell membrane and muscle of each individual were used as samples. We detected serum transferrin (Tf) type and serum esterase (Es) type using horizontal polyacrylamide gel electrophoresis (HPAGE), Hb type was detected using isoelectric focusing (IEF) and blood cell membrane (Cell X) type and muscular protein (Mu) type were detected using

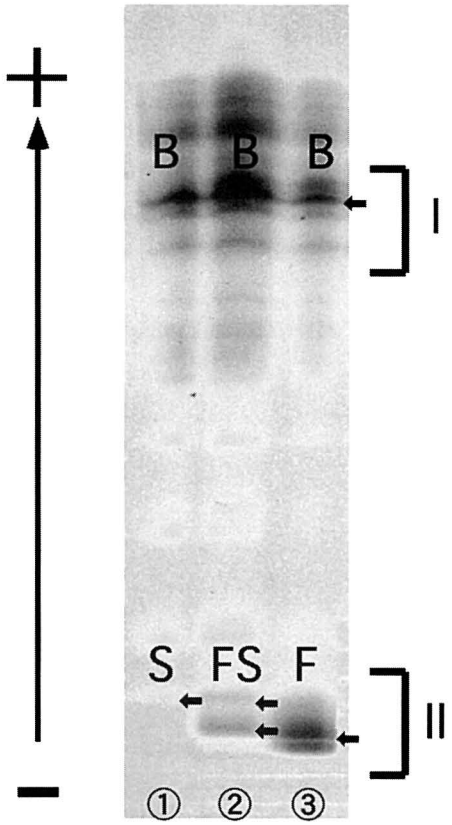


Fig. 2 Electrophoretic patterns of Hemoglobin (Hb) II type on *S. malma* (IEF)
① and ② are *S. m. miyabei* patterns. ③ is *S. m. malma* pattern

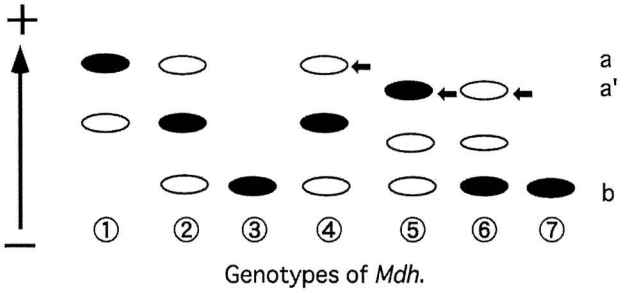
sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[6]. Then we performed Horizontal starch gel electrophoresis to analyze isozyme malate dehydrogenase (MDH ; EC : 1.1.1.37) in muscle and sorbitol dehydrogenase (SDH ; EC : 1.1.1.14) in the liver using CT buffer, pH 7.0[7] and TBCL buffer, pH 8.0[8], respectively.

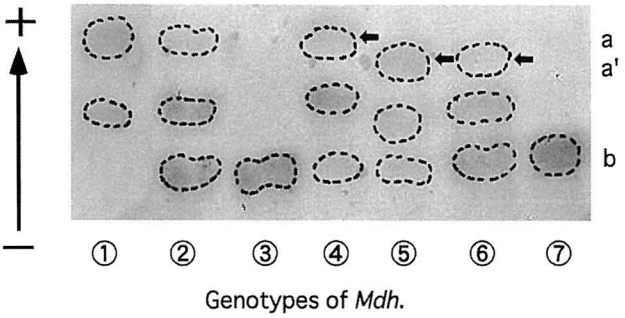
We calculated the proportion of polymorphic loci (*Ppoly*) from allele frequency in each population in which the maximum allele frequency is under 0.95, average heterozygosity ($\bar{H}$) and genetic distance (D)[9]. We constructed the dendrogram from the matrix of genetic distance using the UPGMA method to estimate genetic relationship among the populations.

Results and Discussion

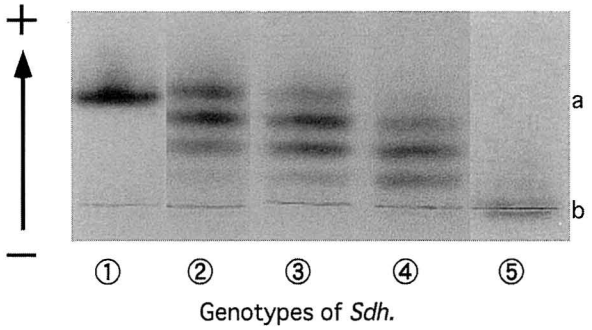
(1) Variability of *S. m. miyabei* and proportion of the polymorphic loci (*Ppoly*) and average heterozygo-sity ($\bar{H}$)

From the result of each protein type analysis and isozyme analysis about MDH and SDH type, we searched 11 kinds of loci (Table 2). In the Shikaribetsu Lake population (*S. m. miyabei*), an S-band was detected in *Hb-II* locus that it was not detected in *S. m. malma*. Furthermore, in the Shikaribetsu Lake population, the a′ band detected in *MDH* locus showed less mobility than the band detected in *S. m. malma* (Fig. 2, 3). Thus,





①, aaaa ②,aabb ③,bbbb ④,aabb ⑤,a'a'a'b
⑥,a'bbb ⑦,bbbb
① - ④ and ⑦ patterns are detected in *S. m. malma*.
⑤ - ⑦ patterns are detected in *S. m. miyabei*.



①,aaaa ②,aaab ③,aabb ④,abbb ⑤,bbbb
① - ④ patterns are detected in *S. m. malma*.
② - ④ patterns are detected in *S. m. miyabei*.
⑤ pattern is detected in *S. leucomaenis*.

Fig. 3 Electrophoretic patterns of MDH in muscle and SDH in liver of *Salvelinus malma malma, S. m. miyabei* and *S. leucomaenis*

Table 3 Nei's genetic distances among 3 populations of *Salvelinus malma* based on 11 loci

	Lake Shikaribetsu (*S. m. miyabei*)	Tokachi River water system (*S. m. malma*)	Other rivers (*S. m. malma*)
Lake Shikaribetsu (*S. m. miyabei*)	-		
Tokachi River water system (*S. m. malma*)	0.163	-	
Other rivers (*S. m. malma*)	0.159	0.017	

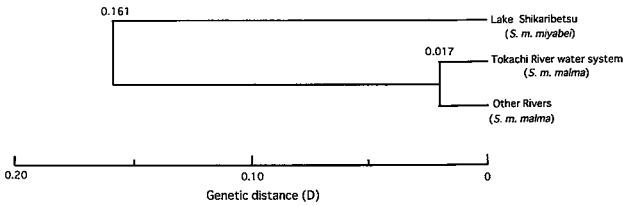


Fig. 4 UPGMA phenogram among *Salvelinus malma* populations in Hokkaido, based on Nei's genetic distances (D) from allele frequencies at 11 loci

the differentiations at the molecular level which were characteristic of *S. m. miyabei* were confirmed in 3 loci including the Pgm type which was reported by Mitsuboshi (1992). In comparison with *S. leucomaenis*, differences that could distinguish the species in the *Tf, Es-I, Es-II, Es-III, Hb-I* and *Mu* loci were detected. Therefore, genetic difference among *S. m. miyabei, S. m. malma* and *S. leucomaenis* were clarified by protein polymorphism and isozyme mutation (unpublished). Of the 11 loci detected, the polymorphism of the Shikaribetsu Lake population (*S. m. miyabei*), Tokachi River water system population (*S. m. malma*) and the other region populations (*S. m. malma*) were detected in 5, 5 and 7 loci respectively, and proportion of polymorphic loci (*Ppoly*) of those populations were 0.455, 0.455 and 0.636 respectively, while the average heterozygosities ($\overline{H}$) were 0.153, 0.217 and 0.219 respectively (Table 2).

(2) The genetic differentiation among 3 *Salvelinus malma* populations

Genetic distances (D) based on 11 loci were compared among the Shikaribetsu Lake population, the Tokachi River water system population and the other regional populations. Genetic distance (D) between the Shikaribetsu Lake population and the Tokachi River water system population was slightly greater than the genetic distance (D) between the Shikaribetsu Lake population and other regional populations (Table 3). And, as a result of making the dendrogram using UPGMA, the Tokachi River water system population and the other region population were connected first in D=0.017, next these 2 groups and Shikaribetsu Lake groups were connected in D=0.161 (Fig. 4). From those results, *S. m. miyabei* showed differentiation greater than the subspecies level (D=0.1)[10] in comparison with *S. m. malma*. Therefore, those findings support the present classification[2,3] that *S. m. miyabei* is a subspecies of *S. m. malma* from the morphological mutation of the number of gill-rakers.

To further clarify the genetic differentiation between *S. m. miyabei* and *S. m. malma*, we should carry out protein analysis, immunological scientific analysis, DNA analysis and isozyme analysis.

Acknowledgment

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ミヤベイワナ（*Salvelinus malma miyabei*）とオショロコマ（*Salvelinus malma malma*）の遺伝的分化

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要約：北海道の然別湖に生息するミヤベイワナはオショロコマの亜種とみなされている。そのミヤベイワナとオショロコマの分化レベルを明確にするために，ミヤベイワナ，然別湖に近い十勝川水系のオショロコマ集団およびその他の河川のオショロコマ集団の3集団に分けて，タンパク質多型およびアイソザイム変異を用いて遺伝的差異および遺伝的分化を検索した。

検索した11種類の遺伝子座のうち，然別湖集団（ミヤベイワナ）に*Hb-II*遺伝子座位および*MDH*遺伝子座位においてそれぞれ*S*バンドおよび*a'*バンドが検出された。これらはオショロコマには検出されない新たなる変異であった。したがって，ミヤベイワナとオショロコマとの間を明確に区別できる遺伝子座位が新たに2座位明らかとなった。また，11種類の遺伝子座の対立遺伝子頻度から集団間の遺伝距離を求め系統樹を作成した結果，十勝川水系の河川の集団（オショロコマ）とその他の地域の河川の集団（オショロコマ）が$D=0.017$ではじめに結びつき，次にこれらの2集団と然別湖集団が$D=0.161$で結びついた。したがって，ミヤベイワナはオショロコマと亜種として明確に位置づけられた。

キーワード：イワナ，タンパク質多型，アイソザイム変異，遺伝的差異，UPGMA法

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