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Genetic Relationships of Oshorokoma Charr (*Salvelinus malma kraschennikovi*), Distributed in Hokkaido, Japan — 1. Central and Eastern Areas —

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Abstract. Genetic diversities and relationships were examined among 'Oshorokoma' charr, *Salvelinus malma kraschenninnikovi*, distributed in the rivers flowing into the Sea of Japan, the Okhotsk Sea, Nemuro Strait and the Pacific Ocean, in Hokkaido, Japan, based on DNA sequences of the cytochrome *b* region of mitochondrial DNA. A total of 6 haplotypes was recognized. There were 5 polymorphic sites in a 500 bp fragment. Oshorokoma was still more distant from *S. leucomaenis* than from *S. alpinus* or *S. fontinalis* in the genetic tree. Haplotype 2, distributed only in the Churui River, was estimated to be the oldest lineage. Haplotype 5 was the most dominant and was not distributed in the rivers flowing into Nemuro Strait. The average genetic distance was 0.003 for the Yubetsu River whereas it was only 0.001 for the Tokachi and Ishikari Rivers. Besides, it was only 0.003 between the rivers originating from the Taisetsu and Shari mountain masses. There was a significant positive correlation between geographical and genetic distances. These results suggest a westward invasion of an ancestral Oshorokoma population, a high genetic diversity in some populations, and a low genetic differentiation among populations. Further studies, including a survey in the whole of Hokkaido, and also including Rishiri and Rebun Islands, are considered to be necessary in the future.

Introduction

Two species of charr, *Salvelinus leucomaenis leucomaenis* (Pallas) (called 'Amemasu'); *S. malma kraschenninnikovi* Taranetz (called 'Oshorokoma'), are distributed in Hokkaido, Japan (Hosoya, 2000). In Hokkaido, some rivers flow into the Sea of Japan, some flow into the Ohotsuk Sea, some flow into the Nemuro Strait and some flow into the Pacific Ocean. The distribution of Oshorokoma is limited; mountain chains or masses of the Shiretoko, Taisetsu, Tokachi, Hidaka and Shakotan. Most individuals in Hokkaido are considered not to migrate seawards, although an anadromous type has been also captured in the rivers originating from the Shiretoko Peninnsula (Saito, 2004). Hamada *et al.* (1998) suggested a sister relationship between *S. malma* and *S. alpinus* based on analysis of *Fok* I family of short interspersed repetitive elements (SINEs). Oleinik *et al.* (2014) examined 27 Alaskan and Asian populations of *S. malma malma* based on mtDNA sequence, recorded 76 haplotypes in 436 fish, and reported weak spatial differences and low levels of divergence. Based on a genetic research of Oshorokoma in 11 rivers in Hokkaido, genetic differences are high among the populations whereas it is extremely low within the population (Saito, 2004). If they remain in rivers, there may be some genetic separations among the populations during every interglacial period. However, genetic structures and relationships of Oshorokoma in

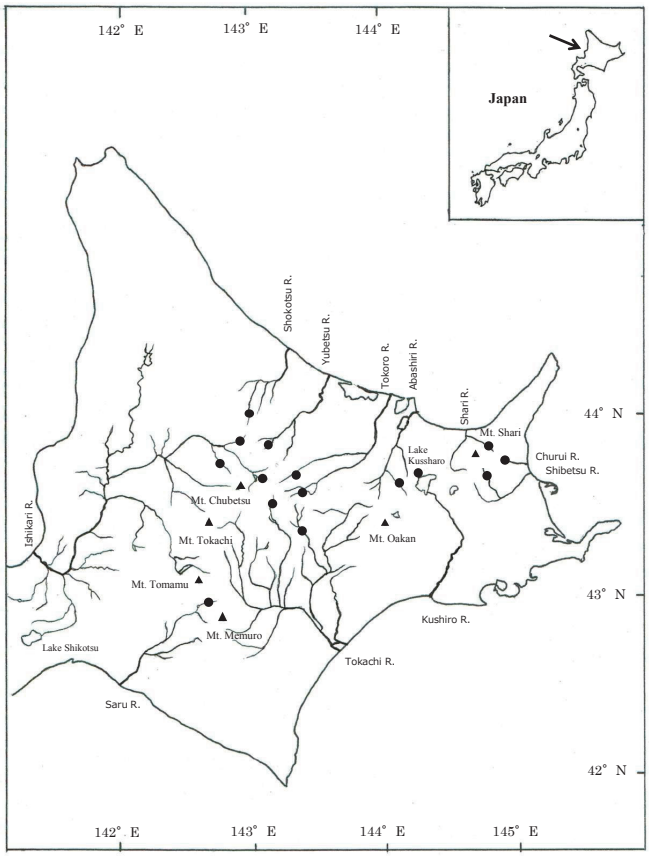

Fig. 1. Map of 14 sampling sites in 11 rivers in Hokkaido.

Hokkaido still remain to be clarified.

In this study, Oshorokoma samples were collected from some rivers flowing into different seas, determined for the DNA sequences in a mitochondrial region, genetic distance was calculated between individuals, and a genetic tree was constructed. Further, genetic distances and relationships were discussed in relation to geographical viewpoints.

Materials and methods

Samples

Charr samples were collected at 14 sites in 11 rivers flowing into the Sea of Japan, the Ohotsuk Sea, the Nemuro Strait and the Pacific Ocean (Fig. 1).

Sampling was performed by fishing using earthworm as a main bait. Samples were transported to the laboratory as a live form using a potable aeration system. After killing by bleeding, samples were measured for body size. Fin was cut, liver was dissected out and both stored in Eppendorf tubes at -30°C until use.

PCR

Template DNA was prepared from the samples using DNeasy Tissue Kit (Qiagen, Tokyo, Japan), according to the manufacturer's instruction.

The cytochrome *b* region of mitochondrial DNA was partially amplified by PCR with a mixture of a template DNA (50 ng) and primers H15915 (5'-ACCTCCGATCTYCGGATTACAAGAC-3'; Aoyama *et al.*, 2000) and L15285 (5'-CCCTAACCGGVTTCTTYGC-3'; Inoue *et al.*, 2000) by using the TaKaRa PCR Amplification kit (TaKaRa, Ohtsu, Japan) in a thermal cycler (Mastercycler personal; Eppendorf, Hamburg, Germany) using the following protocol: preheating at 94°C for 11 min, followed by 30 cycles of denaturation at 94°C for 30 s→annealing at 55°C for 30 s→extension at 72°C for 1 min and a final extension at 72°C for 7 min. PCR products was purified using NucleoSpin Gel and PCR cleanup (Takara, Ohtsu, Japan).

Sequencing was performed directly with the Genetic Analyzer 3130xl (Applied Biosystem, CA, USA) in the Genetic Research Center of Hiroshima University.

Genetic distance

Genetic distance was determined as Tajima-Nei-patameter using MEGA 6.

Dendrogram

Alignment was performed by Clustal W (Thompson *et al.*, 1994) and a genetic tree was constructed by NJ methods using Kimura-2 parameter as a distance by MEGA 6.

Results

A total of 27 samples were collected. Total and body length was in the range of 10.0-20.5 and 8.5-17.5 cm, respectively. Body weight was in the range of 16.0-95.2 g.

Haplotyping

A total of 6 haplotypes were recognized (Table 1). There were 5 polymorphic sites in a 500bp fragment.

Table 1. Sequence variation of 6 haplotypes

Haplotype	29	150	177	235	319
1	C	C	A	G	G
2	C	C	T	G	A
3	C	C	A	A	G
4	T	C	A	G	A
5	C	T	A	G	G
6	C	C	A	G	A

Genetic relationship among 6 haplotypes

Oshorokoma was still more distant from *S. leucomaenis* than *S. alpinus*, and closest to *S. fontinalis*. Haplotype 2 was estimated to be the oldest lineage whereas Haplotypes 3, 5 and 6 were estimated to be new lineages (Fig. 2).

Haplotype composition

Haplotype 5 was the dominant and was not distributed in the rivers flowing into the Nemuro Strait (Table 2). Haplotypes 2, 4 and 6 were distributed only in the Churui, Yubetsu and Ishikari Rivers, respectively. Haplotype 1 was distributed in the Shibetsu and Saru Rivers whereas Haplotype 3 was distributed only in the Ishikari and Yubetsu Rivers.

Genetic distances within and between rivers

The average genetic distance within the samples was 0.003 for the Yubetsu River (Table 3). It was only 0.001 for the Tokachi and Ishikari Rivers. It was 0 for the other rivers. The samples in the Yubetsu River showed relatively high average distances from those in any other rivers. An average genetic distance between the sam-

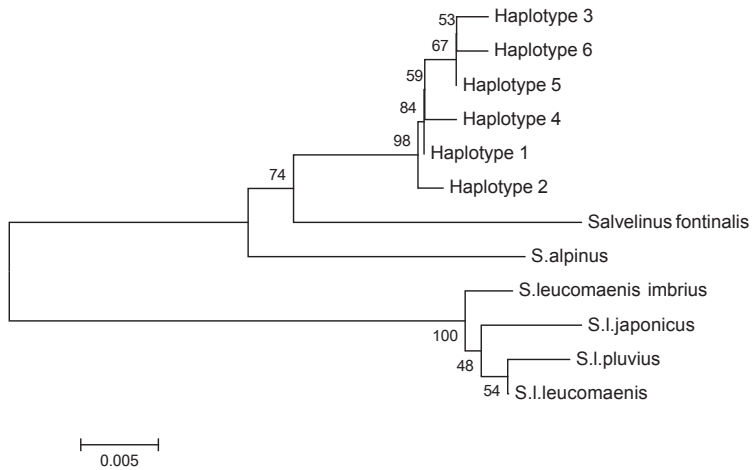

Fig. 2. Genetic relationship of 6 haplotypes.
S. fontinalis, *S. alpinus* and 4 subspecies of *S. leucomaenis* were used as outgroups.

Table2. Haplotype compositions for 11 river basins

Haplotype	River basin											Total
	Sea of Japan	Ohotsuku Sea					Nemuro Strait		Pacific Ocean			
	Ishikari	Shokotsu	Yubetsu	Tokoro	Abashiri	Shari	Churui	Shibetsu	Kushiro	Tokachi	Saru	
1								1			1	2
2							1					1
3	1		2									3
4			3									3
5	6	2		2	2	1			1	3		17
6	1											1
Total	8	2	5	2	2	1	1	1	1	3	1	27

Table 3. Average genetic distances within and between river basins

River	Taisetsu					Shari			Hidaka	Akan	
	Shokotsu	Yubetsu	Tokoro	Ishikari	Tokachi	Shari	Churui	Shibetsu	Saru	Abashiri	Kushiro
Shokotsu	0										
Yubetsu	0.004	0.003									
Tokoro	0	0.004	0								
Ishikari	0.001	0.003	0.001	0.001							
Tokachi	0.001	0.003	0.001	0.001	0.001						
Shari	0	0.004	0	0.001	0.001	ND ※					
Churui	0.004	0.005	0.004	0.005	0.005	0.004	ND				
Shibetsu	0.002	0.003	0.002	0.003	0.003	0.002	0.002	ND			
Saru	0.002	0.003	0.002	0.003	0.003	0.002	0.002	0	ND		
Abashiri	0	0.004	0	0.001	0.001	0	0.004	0.002	0.002	0	
Kushiro	0	0.004	0	0.001	0.001	0	0.004	0.002	0.002	0	ND

※ ND means 'not determined'.

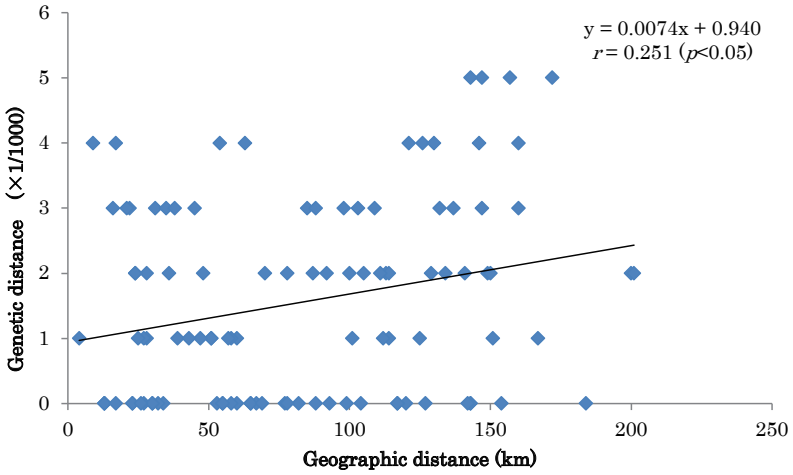

Fig. 3. Relationship between geographical and genetic distance.

ples of the rivers originating from the Taisetsu mountain mass; Shokotsu, Yubetsu, Tokoro, Tokachi and Ishikari Rivers, was only 0.002, in spite of as many samples as 20 individuals in total. In contrast, it was 0.003 between the samples of the rivers originating from the Shari mountain mass; Shari, Shibetsu and Churui Rivers, in spite of only 3 individuals in total. It was only 0.003 between the rivers originating from the Taisetsu and Shari mountain masses.

Relatiopnships between geographical and genetic distances

There was a significant positive correlation between geographical and genetic distances (Fig. 3)

Discussion

In this study, there was a significant positive correlation between geographical and genetic distances of Oshorokoma samples. This suggests some levels of genetic exchanges between adjacent populations taking advantage of some geological events or topographical conditions. Kikko *et al.* (2008) suggested that white-spotted charr dispersed into the northern inlet rivers of Lake Biwa from adjacent inlet rivers of the Sea of Japan by watershed exchanges in the glacial periods of the Pleistocene. Our previous study also showed a possibility of invasion of a charr subspecies, *S. leucomaenis pluvius*, from the Sea of Japan side to the Seto Inland Sea side, taking advantage of river capture (Kawai *et al.*, 2006).

In this study, Oshorokoma was still more distant from *S. leucomaenis* than *S. alpinus*, and closest to *S. fontinalis*. In contrast, Numachi (1975) demonstrated that *S. fontinalis* is the oldest lineage among the species of genus *Salvelinus*, based on isozyme analysis. This discrepancy should be settled by additional studies with native samples of *S. fontinalis*, because a *S. fontinalis* sample used in this study was obtained from an ornamental fish shop.

The origin of Oshorokoma distributed in the rivers of Hokkiado remains unknown. Haplotype 2, collected only in the Churui River, originating from the Shiretoko Mountain Chains, was estimated to be the oldest lineage in the genetic tree whereas Haplotypes 3, 5 and 6, collected in the rivers flowing into the Sea of Japan, Ohotsuku Sea or Pacific Ocean, were es-

timated to be new lineages. This suggests that an ancestral population to Oshorokoma had first invaded into the rivers in the Shiretoko Penninsula. However, the samples from the northern areas, e.g., those from the Teshio River Basin and from the rivers in the Rishiri and Rebun Islands, should also be included before conclusion. There have been no records of Oshorokoma in the Rebun Island, although it has been collected in the Rishiri Island (Shiraishi, 1993)

From the view point of mountain masses, an average genetic distance between the samples of the rivers originating from the Taisetsu mountain mass; Shokotsu, Yubetsu, Tokoro, Tokachi and Ishikari, was only 0.002 in spite of as many samples as 20 individuals in total. This is in accord with the reports in Saito (2004) showing an extremely low genetic diversity within the population. In contrast, it was 0.003 between the samples of the rivers originating from the Shari mountain mass; Shari, Shibetsu and Churui in spite of only 3 individuals in total. Furthermore, it was only 0.003 between the rivers originating from the Taisetsu and Shari mountain masses. This is not in accord with the results in Saito (2004) showing high genetic diversities among populations. On the other hand, Yamamoto (2004) reported the DNA sequence of cyt.*b* for a haplotype of *S. malma* in Hokkaido. Based on DDBJ, this type was genetically very close to but slightly different from our Haplotype 3. Further studies are necessary for clarification of the genetic structure of Oshorokoma, using much more samples, including Alaskan and Asian samples of *S. malma malma*, from as many river basins as possible.

The samples of the Yubetsu River showed relatively high distances from those of any other river basins for some reasons. This might result from a level of hybridization with *S. fontinalis* although no confirmation by appearance. Indeed, we collected some *S. fontinalis* samples from the river basins in our preliminary studies. Besides, hybrid individuals between *S. leucomaenis pluvius* and *S. fontinalis* (called 'Iwakawa') have been a problem due to their fertility in some regions with high altitude (Ida & Okuyama, 2012).

Oshorokoma is also considered to be distributed in southern Hokkaido; the rivers originating from the Kariba Mountains in the Hiyama Region (Saito, 2004) and those from the Mt. Yotei in the Shiribeshi Region (Shiraishi, 1993). Few studies were conducted to examine genetic structures or differences of Oshorokoma in these areas. The populations in these areas are estimated to be isolated from those in main distribution areas such as the Taisetsu Mountains. Therefore, much more complete studies should be conducted on genetic differences among Oshorokoma populations in all the distribution areas of Hokkaido before an extinction due to some effects of global warming.

References

Aoyama, J., S. Watanabe, S. Ishikawa, M. Nishida & K. Tsukamoto, 2000. Are morphological characters distinctive enough to discriminate between two species of freshwater eels, *Anguilla selebesensis* and *A. interiolis*? *Icthyol. Res.*, 47: 157–161.

Hamada, M., N. Takasaki, J. D. Reist, A. L. DeCicco, A. Goto & N. Okada, 1998. Detection of the ongoing sorting of ancestrally polymorphic SINEs toward fixation or loss in population of two species of charr during speciation. *Genetics*, 150: 301–311.

Hosoya, K., 2000. Salmonidae. In "Nihonsan gyorui kensaku" Ed by Nakabo T, pp256–261, Tokai University Press, Tokyo (in Japanese)

Ida, H. & F. Okuyama, 2012. Salmon, trout and char. Yama to Keikokusha, Tokyo, 247pp. (in Japanese)

Inoue, JG., M. Miya, K. Tsukamoto & M. Nishida, 2000. Complete mitochondrial DNA sequence of the Japanese sardine *Sardinops melanostictus*. *Fish. Sci.*, 66: 924–932.

Kawai, K., T. Katayama & H. Imabayashi, 2006. Genetic relationships between chars distributed around the watershed borders in the eastern Chugoku Mountains, Japan, on the basis of RAPD analysis. *J. Grad. Sch. Biosp. Sci.*, 45:1–8.

Kikko, T., M. Kuwahara, K. Iguchi, S. Kurumi, S. Yamamoto, Y. Kai & K. Nakayama, 2008. Mitochondrial DNA population structure of the white-spotted charr (*Salvelinus leucomaenis*) in the Lake Biwa water system. *Zool. Sci.*, 25: 146–153.

Numachi, K, 1975. Isozyme. In “Suisangaku Series 9, Biochemical Classification of Fish species Ed by Japanese Society of Fisheries Science, pp49–64, Kouseishakouseikaku, Tokyo (in Japanese)

Saito, T, 2004. Oshorokoma. *Resource Management Center News of Salmon and Trout*, 13: 9–12. (in Japanese)

Shiraishi, K., 1993. Iwana No Kao. Yama To Keikokusha, 191pp. Tokyo (in Japanese)

Oleinik, AG., L. A. Skurikhina, E. I. Bondar & V. A. Brykov, 2014. Phylogeography of northern Dolly Varden *Salvelinus malma malma* based on analysis of mitochondrial DNA. *J. Zoolg. Syst. Ecol. Res.*, 52: 293–304.

Thompson, JD., D. G. Higgins & T. G. Gibson, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penaltiesand weight matrix choice. *Nucleic Acids Res.*, 22: 4673–4680.

Yamamoto, S., K. Morita, S. Kitano, K. Watanabe, I. Koizumi, K. Maekawa & K. Takamura, 2004. Phylogeography of white-spotted charr (*Salvelinus leucomaenis*) inferred from mitochondrial DNA sequences. *Zool. Sci.*, 21: 229–240.

Yoshiyasu, K., 1996. The book of Japanese char. 255pp. Sakufusha, Tokyo (in Japanese).

北海道産オショロコマの遺伝的関係
—1. 中部および東部地域について—

河合幸一郎・斉藤英俊

ミトコンドリアDNAのチトクロム*b*領域塩基配列に基づき，北海道の日本海・オホーツク海・根室海峡・太平洋に注ぐ河川に分布するオショロコマの遺伝的関係を調べた．計６つのハプロタイプに分けられ，500bpの遺伝子断片中５箇所に多型が見られた．遺伝子系統樹では，オショロコマはアメマスよりもむしろ北極イワナやカワマスに近く，忠類川のみで記録されたハプロタイプ２は最も古い系統と推定された．ハプロタイプ５は最も優占的であったが，根室海峡流入河川では見られなかった．一方，水系内の平均遺伝子距離は湧別川水系で0.003，十勝川・石狩川水系で0.001であった．さらに，大雪山塊と斜里山塊とでは，それぞれから発する水系間の平均遺伝子距離はわずか0.003 であった．また，水系間地理的距離と個体間遺伝子距離との間には有意な正の相関が見られた．これらのことは，オショロコマの祖先が東方から分布を広げていったこと，個体群間の遺伝的分化は低いが，遺伝的多様性が高い個体群も存在する可能性があることを示唆する．今後，利尻・礼文島等，離島を含め，北海道全域を網羅した組織的研究が必要である．