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論文 Article

Genetic relationships in *Yamato-iwana*, a subspecies of white-spotted char, *Salvelinus leucomaenis japonicus*

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Abstract: The genetic relationships in a subspecies of white-spotted char, *Salvelinus leucomaenis japonicus* Oshima, distributed in central Japan, were examined based on the DNA sequences in the mitochondrial cytochrome *b* region. A total of 12 haplotypes were recognized; haplotypes 2 and 5 were co-dominant. Two large clades were observed in a genetic tree. The Hidaka, Yahagi, and Ibi rivers constructed only the larger clade, whereas the Yogo and Sagami rivers constructed only the smaller clade. A small clade of haplotypes 3, 8, and 10 comprising the samples from the Hidaka and Yahagi rivers and almost all samples from Kiso River was separated deeply. In addition, haplotype 8 from the Nomugi (1446 m) site was estimated to be the newest lineage. However, there were no significant correlations between geographical and genetic distances. These results suggest that this subspecies has multiple origins, its genetic structure is determined by other factors than the river basin, and geological events were scarcely involved in its range expansion.

Keywords: Char, genetic relationship, cytochrome *b*, *Salvelinus*

Ⅰ. Introduction

Four subspecies of white-spotted char, *Salvelinus leucomaenis* (Pallas) (called 'Iwana'), are known; *S. l. leucomaenis* (Pallas) (called 'Ezoiwana'), *S. l. pluvius* Jordan et McGregor (called 'Nikkoiwana'), *S. l. japonicus* Oshima (called 'Yamatoiwana') and *S. l. imbrius* Jordan et McGregor (called 'Gogi'). Among these, 'Yamatoiwana' is distributed in the rivers of central Japan flowing into the Pacific Ocean and Lake Biwa (Hosoya, 2000). The origin of char distributed in these rivers is controversial and the taxonomic status and distribution limit are also controversial (Oshima, 1961; Inamura & Nakamura, 1962; Imanishi, 1967; Miyaji et al., 1986; Kimura, 1989; Taki et al., 2005). Yamatoiwana is distinguishable from other 3 subspecies in possession of faint reddish spots on the body side and in lack of clear white spots on the whole body (Hosoya, 2000).Yamamoto et al. (2004) examined genetic relationships among 6 Yamatoiwana populations and reported that they constructed 7 clades in a genetic tree. In our previous study, Yamatoiwana was suggested to have heterogenetic origins by allozyme analysis (Kawai et al., 2007). However, there seem no reports on genetic relationships among Yamatoiwana populations based on more extensive researches.

In this study, we collected Yamatoiwana samples in as many river basins as possible from Hidaka to Sagami River and rivers flowing into Lake Biwa on eastern side. The genetic relationships among the char samples were examined based on the DNA sequences in mitochondrial cytochrome *b* region, and was discussed in relation to geographical distance.

Ⅱ. Materials and Methods

1. Samples

Char samples were collected at a total of 20 sites of 10 river basins in the central region of Japan (Fig. 1; Table 1). Char, identified to Yamatoiwana, according to the description in Hosoya (2000), were used for examination.

Sampling was performed by fishing using earthworm as a main bait at as upper reaches as possible for collection of native fish only. Samples were transported to the laboratory as a live form using a potable aeration system. After killing by bleeding, samples were measured for body sizes. A part of caudal fin was cut, liver was dissected out and both were stored in an Eppendorf tube at -20°C until use.

2. PCR

Template DNA was prepared from the samples using DNeasy Tissue Kit (Quiagen, Tokyo, Japan),

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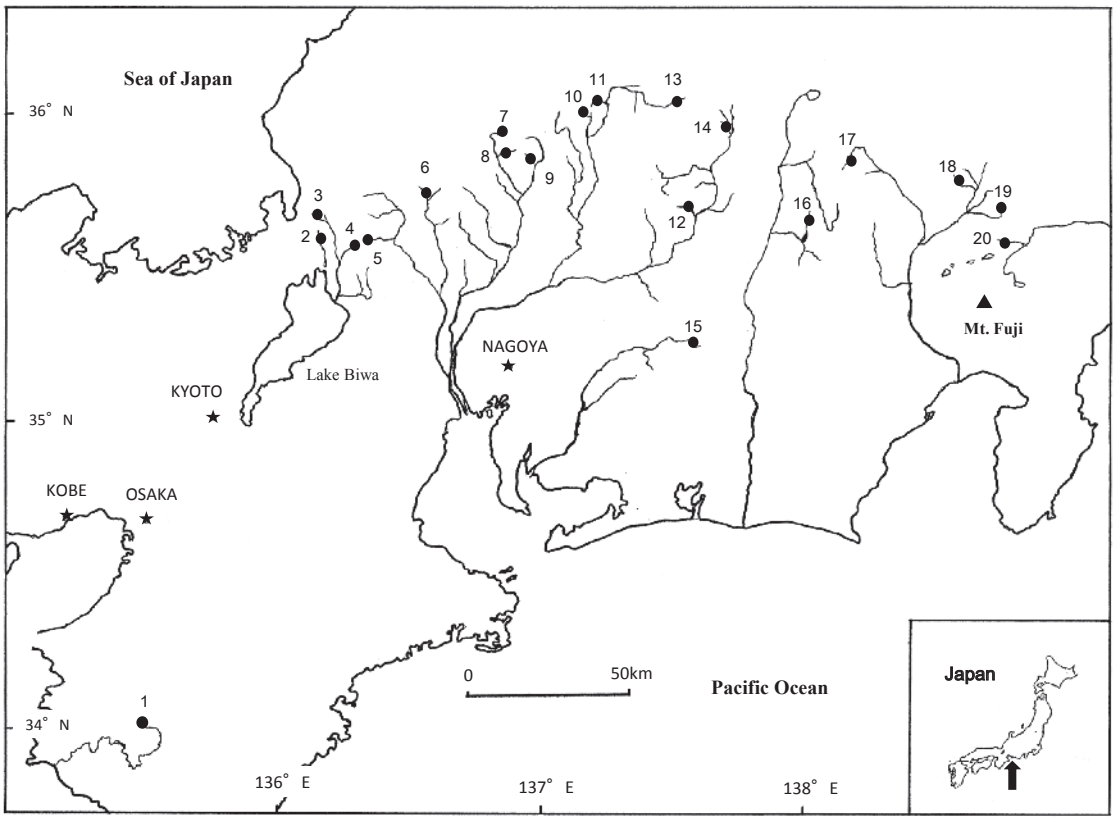

Fig. 1 Map of 20 sampling sites in 10 river basins of the central Japan.

Table 1 Altitude and numbers of samples for 20 sampling sites.

Basin	Site No.	Altitude(m)	No. samples
Hidaka	1	677	1
Yogo	2	379	1
Ane			
Takatoki	3	490	1
Sugino	4	457	2
Ibi			
Asamata	5	564	2
Neo	6	564	2
Nagara			
Washimi	7	996	1
Shiratori	8	913	1
Yoshida	9	971	1
Kiso			
Yamanokuchi	10	1050	1
Sugo	11	867	1
Atera	12	823	1
Nomugi	13	1446	3
Suge	14	1015	1
Yahagi	15	1095	1
Tenryu	16	1336	2
Fuji			
Kamanashi	17	1097	1
Tsuzumi	18	1035	1
Hikawa	19	1337	2
Sagami	20	718	3
Total			29

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'-ACCTC CGATCTYCGGATTACAAGAC-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 were 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.

3. Genetic distance

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

4. Dendrogram

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

Ⅲ. Results

A total of 29 samples were collected. Total and body length was in the range of 9.0-23.0 and 7.5-21.8cm, respectively. Body weight was in the range of 7.0-147.7 g.

1. Haplotyping

A total of 12 haplotypes were recognized (Table 2). There were 14 polymorphic sites in 456bp fragment.

Table 2 Base substitution positions for 12 haplotypes.

Haplotype	Nucleotide position numbers											
	47	64	146	182	194	230	248	275	356	392	416	428	440	452
1	G	G	G	T	A	C	T	C	G	G	T	A	C	C
2	G	G	G	T	A	C	T	T	T	G	C	A	T	C
3	A	G	G	C	G	C	T	C	G	A	T	A	T	C
4	G	G	G	T	A	C	T	C	G	G	C	A	C	C
5	G	G	G	T	A	C	A	C	G	G	C	A	C	C
6	G	T	A	T	A	C	G	T	T	G	C	G	C	C
7	G	G	G	T	A	C	T	C	G	G	C	A	C	T
8	A	G	G	C	G	T	T	C	G	A	T	A	C	C
9	G	G	G	T	A	C	T	T	T	G	C	A	C	C
10	A	G	G	C	G	C	T	C	G	A	T	A	C	C
11	G	G	G	T	A	C	T	C	G	G	A	A	C	T
12	G	G	A	T	A	C	T	T	T	G	C	G	C	C

2. Genetic relationship among 12 haplotypes and haplotype composition

There were 2 large clades; a clade composed of haplotypes 1, 3, 4, 5, 7, 8, 10 and 11 and a clade of 2, 6, 9 and 12. Hidaka, Yahagi and Ibi Rivers constructed the former clade whereas Yogo and Sagami Rivers constructed the latter clade. Other rivers constructed both clades. A small clade of haplotypes 3, 8 and 10 rather deeply separated (Fig. 2). Haplotype 8 was estimated to be the newest lineage.

Haplotypes 2 and 5 were co-dominant and accounted for more than 1/3 of all the samples and comprised samples from as many as 4 and 5 rivers, respectively (Fig. 2). Haplotypes 1, 8 and 12 each comprised samples from a single site. Haplotypes 3, 4, 6, 7, 9 and 11 comprised a single sample.

3. Relatiopnships between geographical and genetic distances

There were no significant correlations between geographical and genetic distances (Fig. 3)

Ⅳ. Discussion

In this study, Yamatoiwana samples were collected in 10 river basins from Hidaka to Sagami River in the central region of Japan, and they were clearly categorized into 2 groups based on genetic differences. This is compatible to our previous results showing much higher *G*st value in Yamatoiwana than those in other two subspecies, Nikkoiwana and Gogi (Kawai et al., 2007). This also supports the results in Yamamoto et al. (2004) showing the highest haplotypic and nucleotide diversities among 4 subspecies. Besides, Kikko et al. (2008) also reported multiple haplotypes in char samples only from the rivers flowing into Lake Biwa in the eastern side where only Yamatoiwana is considered to be distributed.

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. Besides, Katayama and Fujioka (1966) also suggested a possibility of invasion of Gogi from the Takatsu River Basin at the Sea of Japan side to Nishiki River Basin at the Seto Inland Sea side taking advantage of river capture. However, no genetic closeness was recognized between samples from adjacent sites of different rivers in this study; Yogo and Takatoki, Sugino and Asamata, Tenryu and Kamanashi, and Hikawa and Sagami. Thus, watershed exchanges are considered not to be mainly involved in the distribution patterns in this subspecies.

On the other hand, Yamamoto et al. (2004) have reported 10 haplotypes of Yamatoiwana. Among these, 7 haplotypes were not collected in this study. Thus, a total of 19 haplotypes has been recorded to date. Among these, 3 types; Hap3 (Fuji R.), 18 (Ane R.) and 19 (Ane R.) accorded with our Hap.4 (Nagara R.), 12 (Ane R.) and 9 (Yogo R.), respectively. Besides, 9 haplotypes were recorded firstly by this study. An observation of the same haplotypes in the Yogo and Ane River basins may imply a possibility of range expansion of char taking

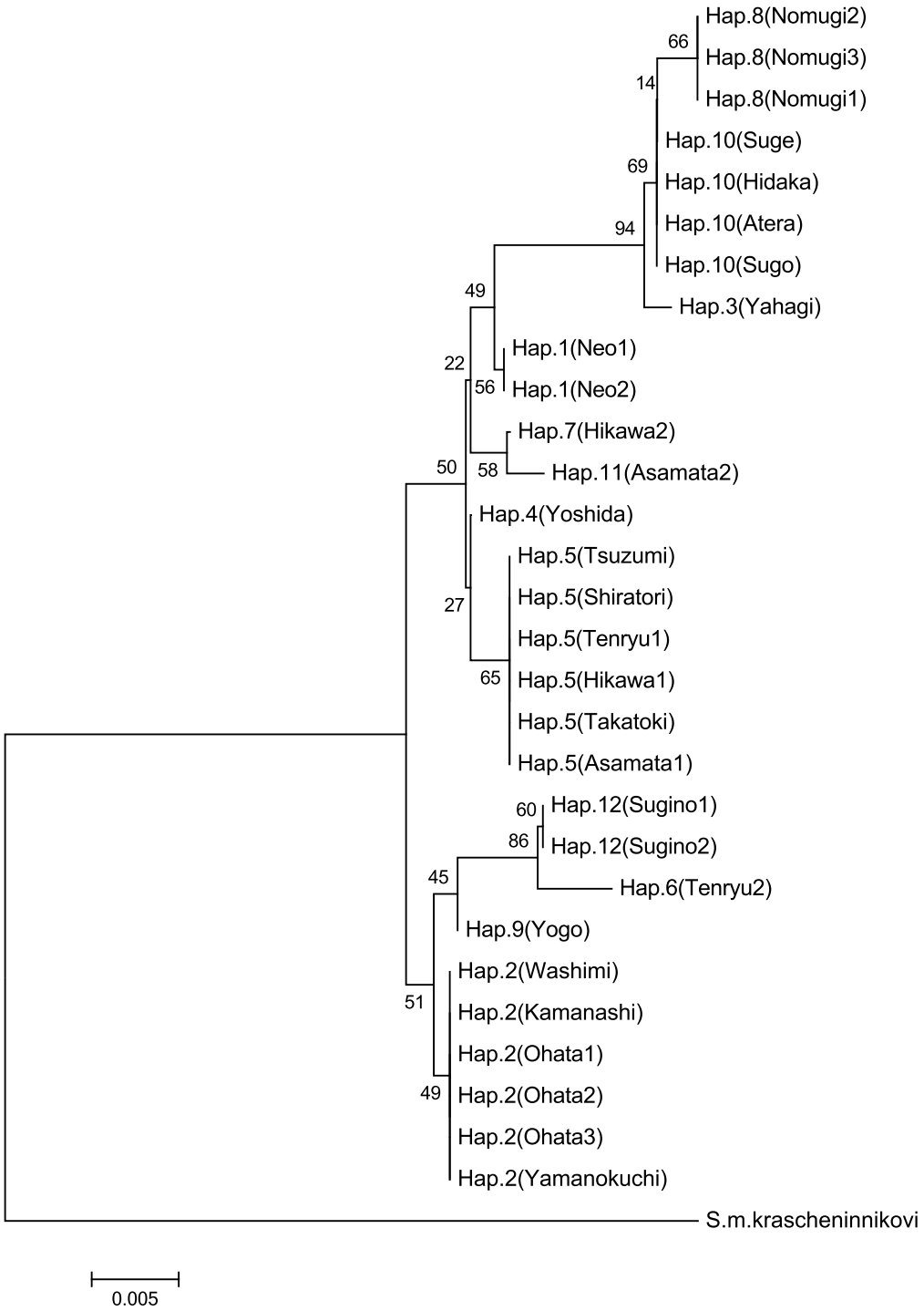

Fig. 2 Genetic relationships among 29 char samples belonging to 12 haplotypes besed on NJ tree constructed by *cyt*.b region sequences in mtDNA. *S. malma krascheninnikovi* was used as an outgroup species.

advantage of watershed exchange, judging from the fact that the headwater of a branch of Ane R., Takatoki R., faces with that of the Yogo River via a pass (Tsubakizaka Pass, 500m). Therefore, further studies are necessary to conclude the contribution of geologic events to range expansion of Yamatoiwana.

Samples from the site Nomugi in the Kiso River Basin were estimated to be the newest lineage in a genetic tree. Considering still the highest altitude of this site among all the sites, genetic isolation is estimated to be an important factor to genetic structures. Besides, a clade composed of haplotypes 3, 8 and 10, and including Hidaka and Yahagi samples and all the samples from the Kiso River Basin excepting the sample from the Yamanokuchi site, was the most deeply separated. This suggests a possibility of some distinct origins to these

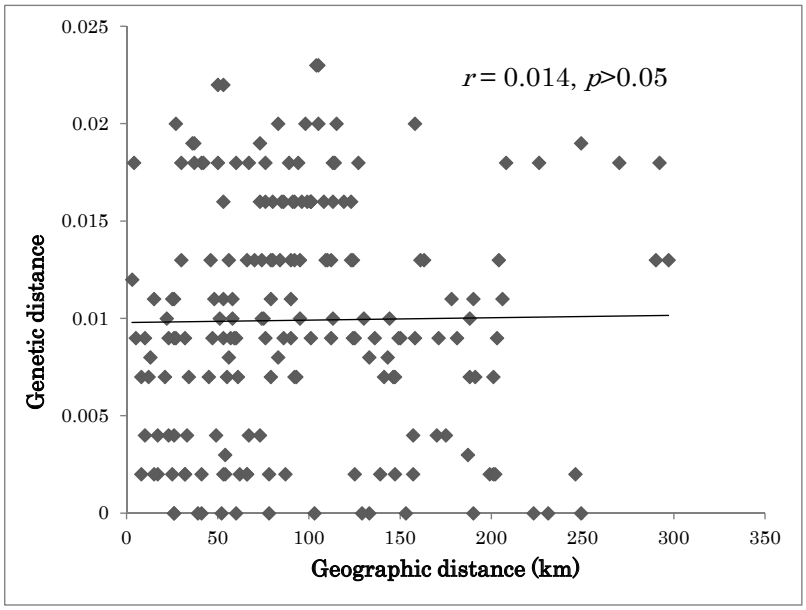

Fig. 3 Relatiopnships between geographical and genetic distances of char samples. Tajima-Nei distance was used as a genetic distance.

haplotypes.

In this study, there were no significant correlations between geographical and genetic distances. This means that neither river basin nor geographical proximity is determinant to genetic closeness. Thus, range expansion of char taking advantage of topographic conditions or geological events is considered to be rather difficult in this central Japan region due to active orogeny in this area.

In this study, a sample from the Hidaka River, originating from the Kii Mountain Chains, was examined. This sample was identified as 'Kirikuchi' based on the characteristic appearance with faint reddish spots and faint parr marks on the body side and almost disappeared whitish spots on the whole body (Shiraishi, 1993). Kirikuchi is considered to be a local population of Yamatoiwana, distributed only in the Kii Peninsula Region. Our result suggests only a morphological variation by Kirikuchi. On the other hand, Yamamoto et. al. (2004) suggests the existence of a genetic clade specific to Kirikuchi. Thus, further study should be carried out with much more samples from the main distribution area, the Kumano River Basin.

Distribution limit of Yamatoiwana is controversial especially for eastern side. Hosoya (2000) described it as the Sagami River, whereas Shiraishi (1993) described it as the Fuji River, western to the Sagami River. Further, some anglers said that the Fuji River Basin has Yamatoiwana in the western branches and has Nikkoiwana in the eastern branches. Therefore, further studies should be undertaken on the whole Fuji Basin to clarify the eastern distribution limit and its meaning.

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