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Genetic relationships between chars distributed around the watershed borders in the eastern Chugoku Mountains, Japan, on the basis of RAPD analysis

Koichiro KAWAI[1)], Takuya KATAYAMA[2)] and Hiromichi IMABAYASHI[1)]

[1)]Graduate School of Biosphere Science, Hiroshima University, Kagamiyama 1-4-4, Higashihiroshima-shi, Hiroshima 739-8528, Japan

[2)]Coca Cola West Japan, Suetake Iwasaki 285-8, Kudamatsu-shi, Yamaguchi 744-0022, Japan

Abstract Genetic relationships were examined among specimens of a subspecies of a Japanese char, *Salvelinus leucomaenis pluvius* ('Nikkoiwana'), distributed around the watershed boundaries in the eastern Chugoku Mountains on the basis of Random Amplified Polymorphic DNA (RAPD) analysis. Thirty seven individuals were collected from 10 branches of 3 river basins. A total of 12 DNA fragments was amplified, among which no fragments were specific to a river basin. All the individuals were categorized into 18 haplotypes on the basis of fragmental combination. Some haplotypes were observed in the two facing branches of different basins across the pass. An extremely high average BSI (Band Similarity Index) was observed between the two adjacent branches around which stream captures had occurred, whereas relatively low BSIs were observed even between the branches within the same basin. In the cluster analysis on the basis of BSI, an intimate cluster was constructed by the adjacent branches of different basins, where the passes were loose on the Japan Sea side. These results suggest that the Nikkoiwana had expanded their distribution ranges from the Japan Sea side to the Seto Inland Sea side taking advantage of highland marshes or geological events such as a stream capture.

Key words: Genetic relationship, Nikkoiwana, RAPD, stream capture, *Salvelinus*.

INTRODUCTION

Two subspecies of a Japanese common char *Salvelinus leucomaenis* (called 'Iwana'), *S. l. pluvius* ('called Nikkoiwana') and *S. l. imbrius* (called 'Gogi'), are distributed in the Chugoku Mountains from which many rivers originate and flow into the Seto Inland Sea or the Japan Sea (Hosoya, 1993). The taxonomic stata of the two subspecies are still controversial (Oshima, 1961; Inamura & Nakamura, 1962; Imanishi, 1967; Miyaji et al., 1986 ; Kimura, 1989). These subspecies are distinguishable in possession of clear white spots on the dorsal surface of the snout by the Gogi (Miyaji et al., 1986; Hosoya, 1993). The distribution boundaries of the two subspecies are also controversial (Imanishi, 1967; Miyaji et al., 1986; Abe, 1987; Kimura, 1989).

Besides, even the origin of the char, distributed in the rivers flowing into the Seto Inland Sea, has not been clarified yet. Yoshiyasu (1996) and Sato (1998) stated that the char had enlarged their distribution areas by artificial stocking. In contrast, Oshima (1961) established a magnificent hypothesis that the Nikkoiwana could have invaded from the Japan Sea side into the Seto Inland

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Sea side by taking advantage of changes in flow cource of rivers resulting from geological events.

In this study, we focused in the Nikkoiwana population distributed in a region of the eastern Chugoku Mountains, not only because multiple branches originate from a mountain mass and flow into the Seto Inland Sea or the Japan Sea but because there is a suggestion of stream captures in the past resulting from a peneplain-like nature in this region (Obata, 1991). Thus, to verify the hypothesis of Oshima (1961), char samples were categorized into haplotypes by Random Amplified Polymorphic DNA (RAPD) analysis, and the relationships between the haplotypes were discussed on the basis of a dendrogram, constructed by use of Band Sharing Index (BSI) in relation to geological factors.

MATERIALS AND METHODS

Research fields and char sampling

We regarded a char distributed in the eastern Chugoku Mountains from the Hino River in the Japan Sea side and from the Yoshii River in the Seto Inland Sea side as the Nikkoiwana according to the description by Miyaji et al. (1986). Thus, we collected char samples in the region of a mountain mass from which many branches of the Sendai (Japan Sea side), Yoshii and Chigusa (Seto Inland Sea side) Rivers originate (Fig. 1).

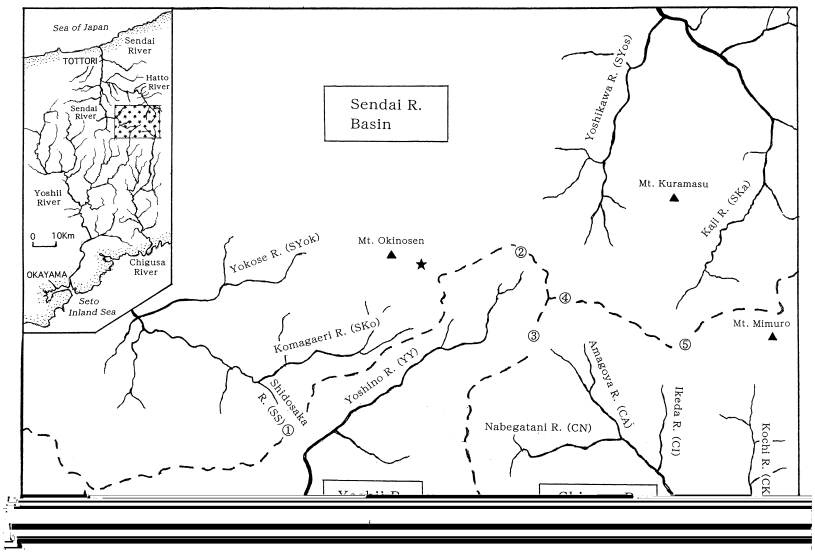

Fig. 1. Map of the research field in the eastern Chugoku Mountains, showing the watershed boundaries between the Sendai (Japan Sea side), Yoshii and Chigusa (both Seto Inland Sea side) Rivers by broken lines.
★The point of wind gap.

We performed a sampling 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, we measured samples for body sizes, dissected the liver out and stored it in an Eppendorf tube at -80℃ until use.

DNA preparation and RAPD analysis

We prepared a DNA template using GenomicPrepTM Cells and Tissue DNA Isolation Kit (Amerscham Biosciences, Piscataway, NJ, USA) according to the manufacturer's instruction.

We used 50ng of prepared DNA as a template. The sequence of an oligonucleotide primer used was 5'-GTAGACCCGT-3'(RAPD Analysis Primer Set 03, Amerscham Biosciences, Piscataway, NJ, USA).

PCR was performed with a DNA thermal cycler (Taitec, Tokyo, Japan) in the following conditions using Ready-To-Go RAPD analysis beads (Amerscham Biosciences, Piscataway, NJ, USA); preheated at 95℃, 1 minute→(95℃, 1 minite→36℃, 1minute→72℃, 2 minutes)×45 cycles→stretched at 72℃, 7 minutes.

Electrophoresis was performed in 1.5% agarose gel at 100 V for 3 hrs. After electrophoresis, gel was stained with ethidium bromide solution.

BSI calculation and dendrogram construction

BSI was calculated according to Lynch (1990) by the following formula;

BSI=2×Nab/(Na+Nb)

where Nab is the number of bands shared by individuals a and b, Na is the number of the bands for individual a and Nb is the number of the bands for individual b.

A dendrogram was constructed by the UPG (Unweighted Pair-Group Clustering) method (Nei, 1975).

RESULTS

A total of 37 samples was collected from 10 branches of 3 rivers. Total length was in the range of 10.5-19.8 cm and body length in the range of 8.8-17.4 cm. Body weight was in the range of 9.5-83.6 g.

RAPD-PCR and haplotyping

A total of 12 DNA fragments was amplified (Fig. 2). Four to eight bands were detected from

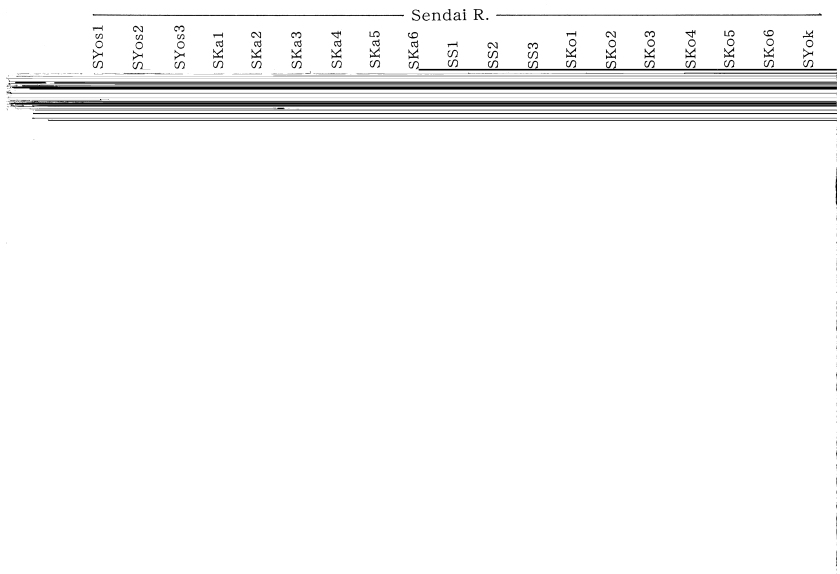

Fig. 2. Schematic presentation of electrophoretic patterns of RAPD-PCR products and haplotyping for 37 individuals from 10 branches of 3 river basins.

an individual. Bands 5, 9 and 10 were common to all the individuals. There were no bands common only to all the individuals of a branch or a river basin.

Eighteen haplotypes were observed (Fig. 2). Hap-1 and -3 were observed only in the Yoshikawa River of the Sendai River Basin. Hap-2 was observed in the Yoshikawa and Kaji Rivers of the Sendai River Basin and the Ikeda River of the Chigusa River Basin. Hap-4 and -5 were observed only in the Kaji River. Hap-6 was observed in the Kaji and Ikeda Rivers. Hap-7 was observed in the Shidosaka and Komagaeri Rivers of the Sendai River Basin and the Yoshino River of the Yoshii River Basin. Hap-8 and Hap-9 were observed only in the Shidosaka River and the Komagaeri and Yokose Rivers of the Sendai River Basin, respectively. Hap-10 was observed in the Yoshino River of the Yoshii River Basin and the Amagoya River of the Chigusa River Basin. Hap-11 and 13 were observed only in the Nebegatani and Kochi Rivers of the Chigusa River Basin, respectively. Hap-12 was observed in the Nebegatani and Kochi Rivers. Hap-14, -15 and -16; and Hap-17 and -18 were observed only in the Ikeda and Amagoya Rivers, respectively.

BSI matrix

A matrix of BSI among 37 samples was shown in Table 1. Average BSI within a branch was usually higher than 0.7 and the highest in SKo (0.97). An average BSI of higher than 0.8 was observed even between the branches of the Sea of Japan side river and the Seto Inland Sea side river: SYos and CA; SKa and CA; SS and YY or CA; SKo and YY, CI or CA; and SYok and YY, CI or CA, whereas an average BSI of lower than 0.70 was observed even between branches of the same river: SYos and SS; and CN and CI or CA.

Table 1. A matrix of BSI among a total of 37 samples from 10 branches of 3 rivers. Top, range; bottom, average.

	SYos (n=3)	SKa (n=6)	SS (n=3)	SKo (n=6)	SYok (n=1)	YY (n=3)	CN (n=3)	CK (n=3)	CI (n=6)	CA (n=3)
SYos	0.77-0.92 (0.84)									
SKa	0.71-1.00 (0.87)	0.77-1.00 (0.91)								
SS	0.62-0.83 (0.69)	0.62-0.91 (0.73)	0.83-1.00 (0.89)							
SKo	0.62-0.83 (0.75)	0.71-0.91 (0.83)	0.83-1.00 (0.89)	0.92-1.00 (0.97)						
SYok	0.62-0.77 (0.70)	0.71-0.83 (0.78)	0.92	0.92-1.00 (0.94)	1.00					
YY	0.67-0.92 (0.70)	0.77-0.93 (0.72)	0.77-1.00 (0.87)	0.86-1.00 (0.96)	0.86-0.92 (0.90)	0.92-1.00 (0.95)				
CN	0.67-0.92 (0.70)	0.60-0.91 (0.77)	0.55-0.83 (0.66)	0.50-0.83 (0.73)	0.50-0.77 (0.68)	0.50-0.83 (0.74)	0.55-1.00 (0.70)			
CK	0.67-0.92 (0.80)	0.67-0.91 (0.79)	0.60-0.83 (0.68)	0.55-0.83 (0.75)	0.55-0.77 (0.69)	0.60-0.83 (0.76)	0.22-1.00 (0.71)	0.60-1.00 (0.73)		
CI	0.67-1.00 (0.70)	0.62-1.00 (0.79)	0.57-0.92 (0.78)	0.57-0.93 (0.83)	0.67-0.93 (0.83)	0.57-0.92 (0.82)	0.31-0.91 (0.63)	0.50-0.91 (0.72)	0.62-1.00 (0.79)	
CA	0.71-0.92 (0.83)	0.77-0.93 (0.87)	0.77-0.92 (0.82)	0.77-0.93 (0.86)	0.86-0.93 (0.88)	0.77-1.00 (0.88)	0.46-0.77 (0.66)	0.67-0.77 (0.74)	0.67-0.92 (0.82)	0.86-0.93 (0.91)

Dendrogram

A dendrogram was constructed on the basis of BSI (Fig. 3). Largely 4 clusters were constructed at a BSI level of 0.8 : Hap-8 (Shidosaka River); Hap-3 and Hap-14 (Yoshikawa and Ikeda Rivers); Hap-11 and Hap-13 (Nabegatani and Kochi Rivers)and other haplotypes (all rivers). Hap-1, -2 and -12 (Yoshikawa, Kaji, Nabegatani, Kochi and Ikeda Rivers); and Hap-6 and -7 (Kaji, Komagaeri, Shidosaka, Yoshino and Ikeda Rivers) each constructed a small intimate cluster. Hap-4, -5, -9, -10, -15~18 (Kaji, Komagaeri, Yokose, Yoshino, Amagoya and Ikeda Rivers) constructed a large intimate cluster.

Geological factors affecting genetic intimacy between the facing branches

The altitude was only 590m for the Shidosaka Pass whereas it was higher than 1000m for all other passes (Table 2). The mean gradient from the pass to the origin (headwater) was the lowest for the Seto Inland Sea side of the Wakasugi. It was moderate for the Seto Inland Sea side of the Enami and the Japan Sea sides of the Shidozaka, Minegoe and Odori. It was high for the Seto Inland Sea sides of the Shidozaka, Minegoe and Odori and the Japan Sea side of the Wakasugi. It was the highest for the Japan Sea side of the Enami.

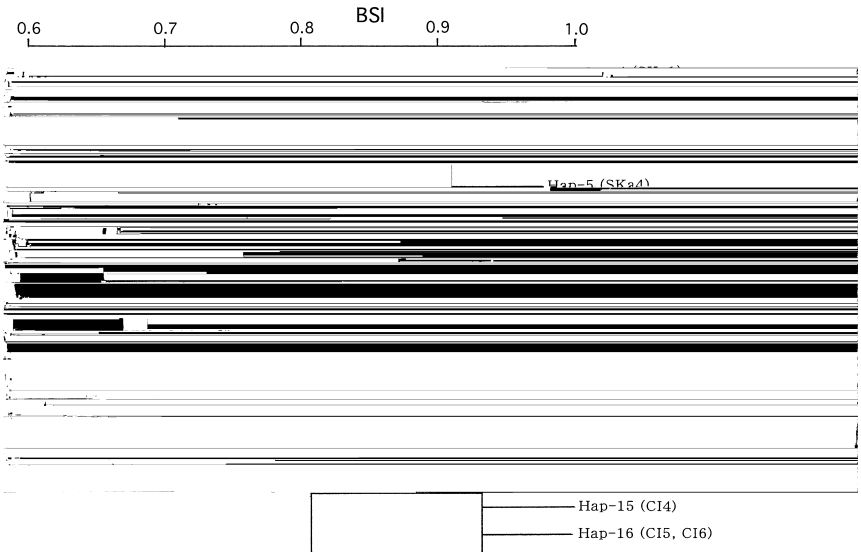

Fig. 3. A dendrogram of 18 haplotypes on the basis of BSI.

Table 2. Altitude of pass and the gradients from the pass to both origins.

Symbol	Pass	Altitude	Gradient (× 1000) to origin*	
			Seto Inland Sea side	Japan Sea side
①	Shidosaka	590	213	122
②	Wakasugi	1050	81	233
③	Minegoe	1020	244[a]	113[b]
④	Enami	1110	138	255
⑤	Odori	1020	240	154

*The mean gradient from the pass to a origin (riverhead in the map of 1:25000).

[a]Chigusa River side, [b]Yoshii River side.

DISCUSSION

The BSI matrix showed relatively low average values (0.69-0.73) between the Yoshikawa or Kaji (small branches of a large branch, the Hatto River) and Shidosaka (a small branch of the main river) Rivers, although all are a tributary to the Sendai River. No identical haplotypes were observed between these combinations of branches. Furthermore, no individuals of the Yoshikawa

(1986).

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RAPD 分析に基づく中国地方東部分水界周辺産イワナの遺伝的関係

河合幸一郎[1)]・片山　拓也[2)]・今林　博道[1)]

[1)]広島大学大学院生物圏科学研究科，東広島市 739-8528
[2)]西日本コカコーラ，下松市　744-0022

要　旨　RAPD 分析により中国地方東部分水界のニッコウイワナ個体間の遺伝的関係を調べた。３水系10支流から37個体が採集され，12の DNA 増幅断片が見られたが，河川特異的断片は見られなかった。全個体は断片の組み合わせによって18のハプロタイプに分けられた。いくつかのハプロタイプは，峠を挟んで接する異なる水系の２支流間で見られた。過去に河川争奪が起こった可能性が示唆されている隣接２支流間で極めて高い平均 BSI 値が見られたのに対し，同水系内の支流間でも比較的低い BSI が見られた。BSI に基づくクラスター分析では，峠付近が日本海側で緩やかになっているような隣接異水系支流間で緊密なクラスターが形成された。これらの結果は，ニッコウイワナが，高層湿原や河川争奪などの地形変動を利用して，日本海側から瀬戸内側へと分布域を拡大してきたことを示唆する。

キーワード：遺伝的関係，イワナ属，河川争奪，ニッコウイワナ，RAPD