論文 Article

Genetic Relationships between 'Gogi', a Subspecies of Japanese Common Charr, *Salvelinus leucomaenis imbrius*, Distributed around the Watershed Borders in the Western Chugoku Mountains, Japan, on the Basis of RAPD Analysis

Koichiro KAWAI[1], Yuji INA[1] and Hiromichi IMABAYASHI[1]

Abstract: Genetic relationships were examined among specimens of a subspecies of the Japanese common charr, *Salvelinus leucomaenis imbrius* (Gogi), distributed around the watershed boundaries in the western Chugoku Mountains on the basis of Random Amplified Polymorphic DNA (RAPD) analysis. A total of 23 fish was collected from 16 branches of 4 rivers. Twenty-one DNA fragments were amplified, among which there were no bands common only to all the individuals of a branch or a river system. A genetical identity was observed among the branches of the two facing river systems across a watershed, the Takatsu and Nishiki River systems. The highest two average Band Sharing Index (BSI) values were also observed between these river systems. Average BSI was the lowest between the Nishiki and Ohta River systems, although their rivermouths are adjacent. BSI between the individuals showed a significantly negative relationship against the distance between the collection sites. Two large clusters comprised the individuals from 3 or all the river systems at BSI level of 0.8. Some intimate clusters were constructed by the facing branches of different river systems. These results suggest that the genetic distance of the Gogi might be strongly determined not by the river system but by the geographical closeness.

Keywords: Genetic relationship, Gogi, RAPD, *Salvelinus*, Stream capture

Ⅰ. Introduction

Two subspecies of the Japanese common charr, *Salvelinus leucomaenis* (Pallas) (called 'Iwana'); *S. l. pluvius* (Hilgendorf) (called 'Nikkoiwana') and *S. l. imbrius* (Jordan et Mc Gregor) (called 'Gogi'), are distributed in the Chugoku Mountains (Hosoya, 2000). The taxonomic stata of the two subspecies are still controversial (Oshima, 1961; Inamura & Nakamura, 1962; Imanishi, 1967; Miyaji et al., 1986; Kimura, 1989). Gogi is distinguishable from the Nikkoiwana in the possession of clear white spots on the dorsal surface of the snout (Miyaji et al., 1986; Hosoya, 2000). Sato (1963) carried out vigorous studies on the morphology, ecology and distribution of Gogi. Although Gogi is distributed in rivers flowing into both the Seto Inland Sea and the Sea of Japan, the origin of Gogi as well as the genetic relationships between both sides is still unknown.

In this study, we focused in the Gogi populations in a boundary region of the watershed in the western Chugoku Mountains, from which multiple branches originate and flow into the Seto Inland Sea or the Sea of Japan, partly because there is a suggestion of stream captures in the past resulting from a peneplain-like nature in this region (Obata, 1991). Thus, charr samples were collected around the boundary region, the genetic distances were measured by band sharing index (BSI) on the basis of Random Amplified Polymorphic DNA (RAPD) analysis, and genetic relationships are discussed on the basis of geographical distance and topographical factors.

Ⅱ. Materials and methods

1. Fish sampling

We regarded charr distributed in the western Chugoku Mountains from the Hii River in the Japan Sea side and from the Ohta River in the Seto Inland Sea side as the Gogi according to the description by Miyaji et al. (1986). Thus, we collected charrs in the region of a mountain chain from which many branches of the Sufu, Takatsu (Sea of Japan side), Ohta and Nishiki (Seto Inland Sea side) River systems originate (Fig. 1).

We made sampling by fishing using earthworm as a main bait at as upper reaches as possible for collection of

1 Graduate School of Biosphere Science, Hiroshima University

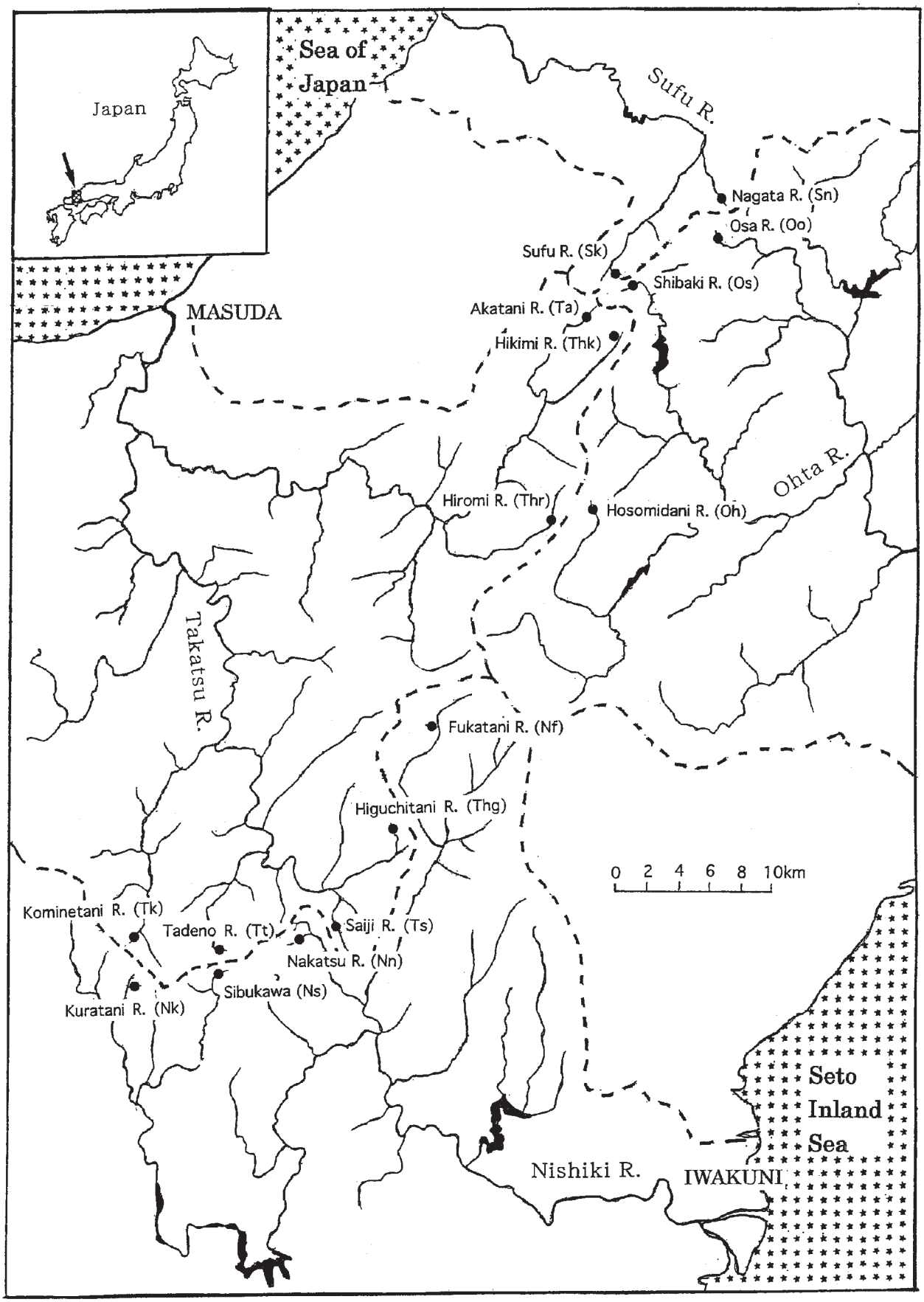

Fig. 1 Map of the research field in the western Chugoku Mountains, showing the sampling sites and watershed boundaries among the Takatsu, Sufu (Sea of Japan side), Nishiki and Ohta (Seto Inland Sea side) Rivers by broken lines.

native fish only. Fish were transported alive to the laboratory using a potable aeration system. After killing by bleeding, we measured them for body sizes, dissected the liver out and stored it in an Eppendorf tube at -80℃ until use.

2. DNA preparation and RAPD analysis

We prepared a DNA template using GenomicPrep™ 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'-GGTGCGGGAA-3' (RAPD Analysis Primer Set 01, Amerscham Biosciences, Piscataway, NJ, USA).

PCR was performed with a DNA thermal cycler (TR-100, 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℃, 1 minute→72℃, 2 minutes)×45 cycles→stretched at 72℃, 7 minutes.
Electrophoresis was performed in 1.5% agarose gel at 100V for 3hrs. After electrophoresis, gel was stained with ethidium bromide solution.

3. BSI calculation and dendrogram construction

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

$$BSI=2\times Nab/(Na+Nb)$$

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).

4. Results

A total of 23 fish was collected from 16 branches of 4 rivers. Total and body lengths ranged from 10.6 to 22.2 cm and from 9.00 to -18.5 cm, respectively. Body weight was 9.60-82.3 g.

5. RAPD-PCR

A total of 21 DNA fragments was amplified (Fig. 2). Nine to18 bands were detected from an individual. Bands 8, 12-16 and 20 were common in all the individuals. There were no bands common only to all the individuals of a branch or a river basin.

6. BSI matrix

A matrix of BSI among 23 individuals was shown in Table 1. A genetic identity was observed among the Higuchitani River of the Takatsu River system, and the Kuratani and Shibukawa Rivers of the Nishiki River system. Average BSI within a river system was the highest (0.87) for the Sufu River system and higher than 0.8 for the Takatsu and Nishiki River systems. It was the lowest (0.76) for the Ohta River system. An average BSI between the basins was 0.79-0.86 in the range and the highest two values were observed between the combinations of the Sea of Japan-facing and the Seto Inland Sea-facing river systems, i.e., the Takatsu and Nishiki River systems and the Sufu and Nishiki River systems. It was the lowest between the Nishiki and Ohta River systems, although

Table 1 A matrix of BSI among a total of 23 samples from 16 branches of 4 rivers.

	Takatsu										Nishiki							Sufu			Ohta	
	Kominetani		Tadeno	Saiji		Higuchidani		Hiromi	Hikimi	Akatani	Kuratani		Shibukawa		Nakatsu		Fukatani	Sufu		Nagata	Hosomidani	Shibaki
	Tk1	Tk2	Tt	Ts1	Ts2	Thg1	Thg2	Thr	Thk	Ta	Nk1	Nk2	Ns1	Ns2	Nn1	Nn2	Nf	Sk1	Sk2	Sn	Oh	Os
Tk2	0.83																					
Tt	0.91	0.85																				
Ts1	0.86	0.80	0.96																			
Ts2	0.80	0.90	0.89	0.85																		
Thg1	0.83	0.93	0.85	0.80	0.97																	
Thg2	0.87	0.96	0.88	0.83	0.93	0.96																
Thr	0.91	0.77	0.83	0.78	0.82	0.85	0.80															
Thk	0.90	0.77	0.91	0.86	0.80	0.83	0.87	0.82														
Ta	0.74	0.84	0.83	0.79	0.88	0.84	0.87	0.69	0.74													
Nk1	0.87	0.96	0.88	0.83	0.93	0.96	1.0	0.80	0.87	0.87												
Nk2	0.95	0.88	0.96	0.91	0.85	0.88	0.92	0.87	0.95	0.79	0.92											
Ns1	0.83	0.86	0.92	0.88	0.90	0.86	0.89	0.77	0.83	0.90	0.89	0.88										
Ns2	0.87	0.96	0.88	0.83	0.93	0.96	1.0	0.80	0.87	0.87	1.0	0.92	0.89									
Nn1	0.80	0.83	0.82	0.77	0.87	0.90	0.86	0.89	0.80	0.81	0.86	0.85	0.90	0.86								
Nn2	0.95	0.78	0.86	0.80	0.75	0.78	0.82	0.86	0.84	0.69	0.82	0.90	0.78	0.82	0.75							
Nf	0.95	0.78	0.86	0.90	0.75	0.78	0.82	0.86	0.84	0.69	0.82	0.90	0.78	0.82	0.75	0.89						
Sk1	0.90	0.75	0.82	0.76	0.72	0.75	0.78	0.82	0.90	0.74	0.78	0.86	0.83	0.78	0.80	0.84	0.84					
Sk2	0.80	0.83	0.82	0.77	0.93	0.90	0.86	0.82	0.72	0.88	0.86	0.77	0.90	0.86	0.87	0.75	0.75	0.80				
Sn	0.87	0.82	0.80	0.75	0.86	0.89	0.85	0.88	0.78	0.80	0.85	0.83	0.89	0.85	0.93	0.82	0.82	0.87	0.93			
Oh	0.91	0.77	0.83	0.78	0.82	0.85	0.80	0.92	0.82	0.76	0.80	0.87	0.85	0.80	0.89	0.86	0.86	0.91	0.89	0.96		
Os	0.70	0.82	0.80	0.75	0.86	0.82	0.85	0.64	0.78	0.87	0.85	0.75	0.82	0.85	0.71	0.64	0.64	0.70	0.79	0.69	0.64	
Oo	0.71	0.81	0.80	0.76	0.91	0.88	0.84	0.73	0.71	0.91	0.84	0.76	0.88	0.84	0.85	0.67	0.67	0.71	0.91	0.84	0.80	0.84

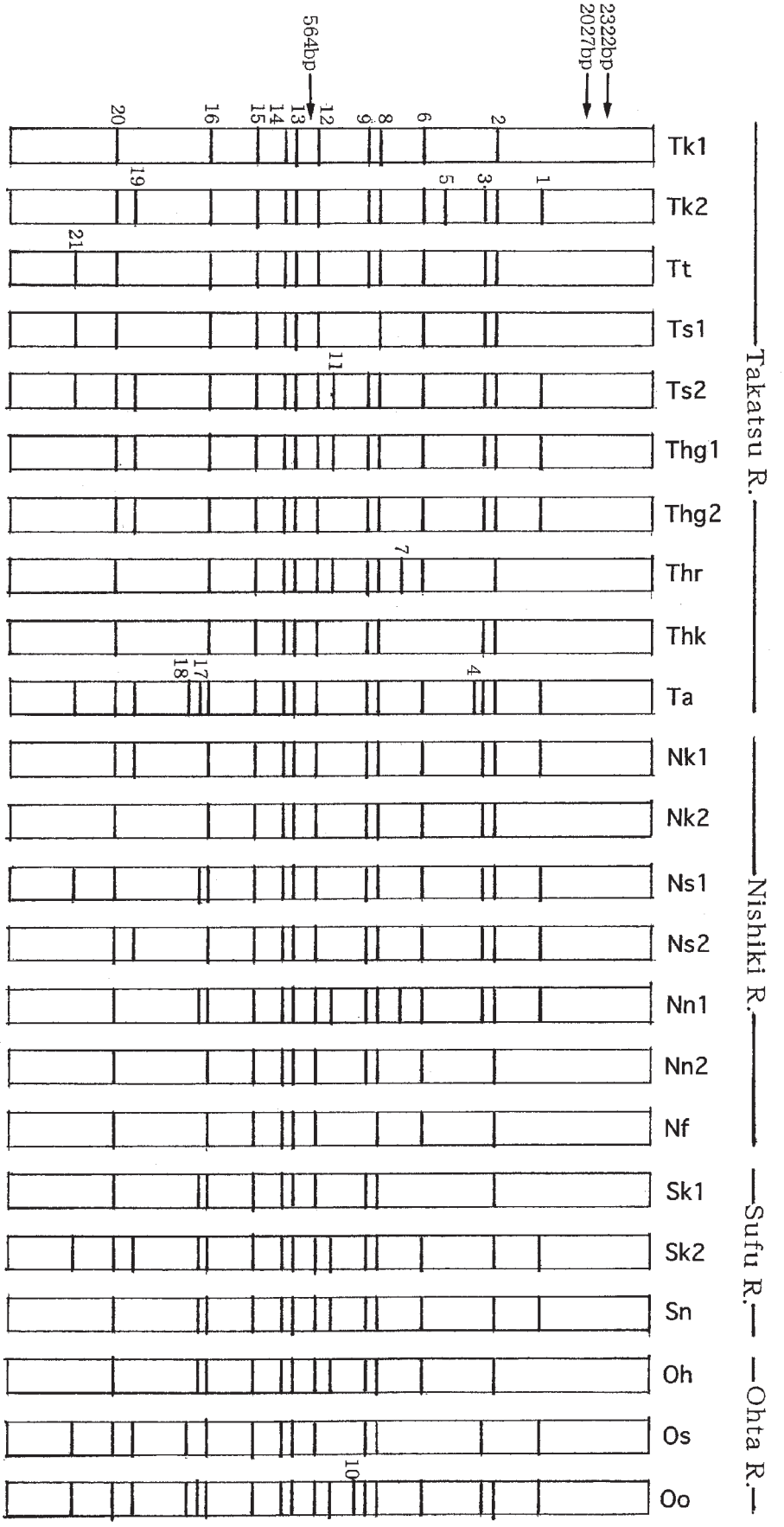

Fig. 2 Schematic presentation of electrophoretic patterns of RAPD-PCR products of 23 individuals from 16 branches of 4 river basins.

their rivermouths are adjacently situated.

7. Relationship between BSI and distance

BSI value between the individuals showed a significantly negative relationship against the distance between the sites where those were collected, although the correlation coefficient was low (Fig. 3).

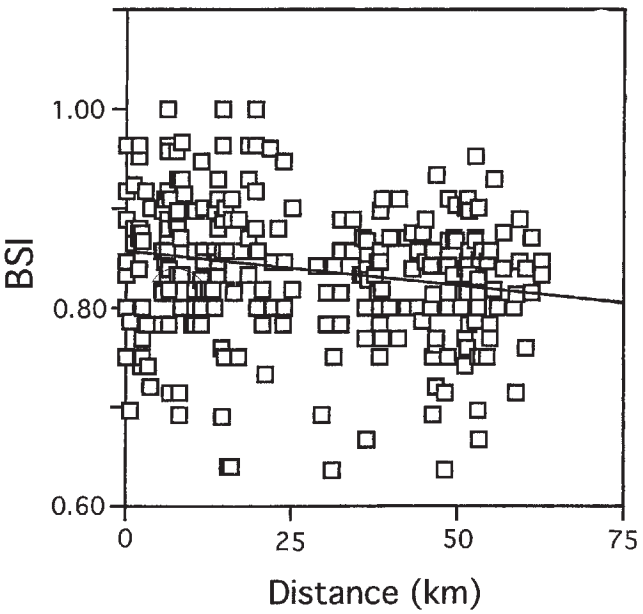

Fig. 3 Relationship of the distance between the collection sites against BSI. (y=0.855−0.00067x, r=−0.187, n=253, p<0.01)

8. Dendrogram

A dendrogram was constructed on the basis of BSI (Fig. 4). Largely 2 clusters were constructed at a BSI level of 0.8 and both comprised the individuals from 3 or all the river systems. Two clusters (Ta, Oo, Ns1; Sn, Oh, Nn1, Sk2, Thr) comprised 3 or all the river systems even at BSI level of >0.87. Only two individuals (Ts2 and Thg1) scarcely constructed an exclusive cluster of a river system at a high BSI level of 0.95. Some intimate clusters were constructed by the geographically close branches from different river systems (Ta and Oo; Tt, Ts1 and Nk2).

Ⅲ. Discussion

In this study, a total of 21 DNA fragments was amplified by RAPD-PCR, among which there were no bands common only to all the individuals of a branch or a river system, indicating an weak reproductive isolation mechanism among river systems. Nevertheless, the isolation level of the Gogi has been reported to be higher than that of the Nikkoiwana (Kawai et al., 2007). The observed low isolation level can be explained by a range expansion via seaward migration, supported by fishing records of anadromous individuals of the Gogi in the

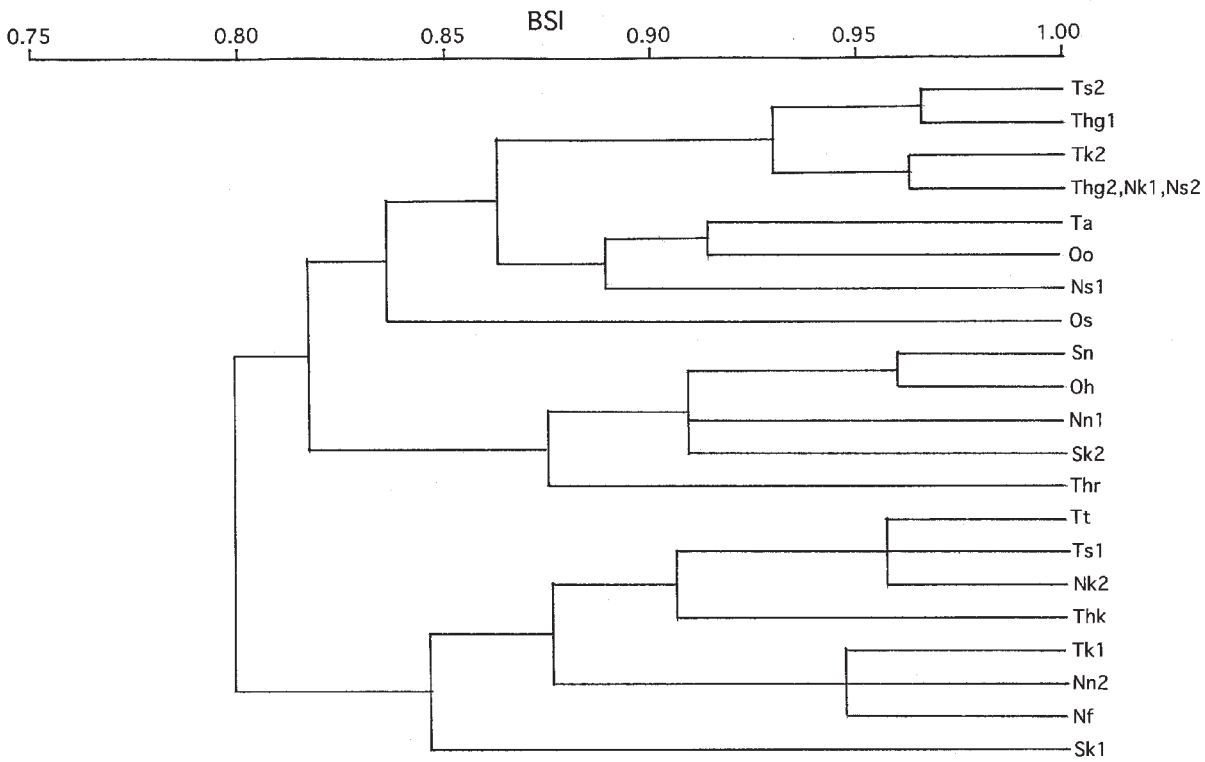

Fig. 4 A dendrogram of 23 individuals on the basis of BSI.

San-in area (Taguchi, 2005) or more possibly by that with exploitation of topographical factors such as stream capture and highland marsh resulting from peneplain-like nature in the western Chugoku Mountain Chains (Obata, 1991).

A genetic identity was observed among the Higuchitani River of the Takatsu River system, and the Kuratani and Shibukawa Rivers of the Nishiki River system. Besides, the highest two average BSIs between the river systems were observed between the combinations of the Sea of Japan-facing and the Seto Inland Sea-facing river systems, i.e., the Takatsu and Nishiki River systems and the Sufu and Nishiki River systems. In the previous study, we also observed the genetic identity between the adjacent branches of a Sea of Japan-facing river system, the Sendai River system and Seto Inland Sea-facing river system, the Yoshii or Chigusa River system in the eastern Chugoku Mountain Chains (Kawai et al., 2006). On the other hand, average BSI was the lowest between the Nishiki and Ohta River systems, although their rivermouths are adjacently situated. These suggest that a river system itself or adjacency of rivermouth should hardly be determinative factor to genetic distance among the Gogi charr. This is also supported by the results of cluster analysis. That is, large 2 clusters were constructed at a BSI level of 0.8; a larger cluster comprised the individuals from all the river systems and another smaller one comprised those from all the river systems excepting the Ohta River system. Besides, two intimate clusters (Ta and Oo; Tt, Ts1 and Nk2) were constructed by the adjacent branches from different river systems. Furthermore, BSI value between the individuals showed a significantly negative relationship against the distance between the sites where those were collected. These results suggest that a possible range expansion mechanism not via seaward migration should be one of the determinative factors to genetic distance. That is, natural events such as geological changes might have been exploited by the Gogi populations for invasion from the Sea of Japan-facing river systems into the Seto Inland Sea-facing ones.

On the other hand, the origin of the charr distributed in the Seto Inland Sea-facing river systems still remains controversial. Oshima (1961) stated that the Nikkoiwana populations had invaded from the Sea of Japan-facing Sendai River system to the Seto Inland Sea-facing Yoshii River system via stream capture and those had further invaded from the Yoshii River system to the Seto Inland Sea-facing Chigusa River system by exploitation of highland marsh. However, Takeshita (1988) compiled many records of the artificial stockings of charr from the Sea of Japan-facing river systems to the Seto Inland Sea-facing river systems in the Chugoku Region by hearing. However, natural range expansion process of the Gogi is not incompatible to artificial stocking for the distribution of charr in the Seto Inland Sea side. Nevertheless, further compilation of artificial stocking records and the examination of genetic identity between the Gogi in the Seto Inland Sea-facing rivers and those in the supposed original river systems need to be carried out before conclusion.

Average BSI within a river system was higher than 0.8 for all the river systems except for the Ohta River system. This might imply multiple origins of the population in the Ohta River system. On the other hand, the Gogi is the secondly southernmost distributed subspecies to ‘Kirikuchi’ (a regional population of ‘Yamatoiwana’, *S. l. japonicus* Oshima) in Japan. Furthermore, the headwater of the Fukugawa River, a branch of the Takatsu River system, where the Gogi is abundant, has been reported to be near to the southern distribution limit of the genus *Salvelinus* in the world (Yasue, 1981). In addition, the Gogi was estimated to be still more primitive subspecies than the Yamatoiwana and Nikkoiwana (Numachi, 1975). Thus, the genetic structure of the Gogi should be clarified in order to maintain a native biological resource to the Chugoku Region.

【Referenes】

Hosoya, K. (2000): Salmonidae. In: Nakabo T (ed.). *Nihonsan gyorui kensaku*, Tokai University Press, Tokyo, 256-261 (in Japanese).

Imanishi, K. (1967): Chars, the genus *Salvelinus*, its distribution in Japan. *Shizen*, 3-46 (in Japanese).

Inamura, A. and Nakamura, M. (1962): Distribution and variation of fishes in the genus *Salvelinus* in Japan. *Rep. Res. Inst. Nat. Resource*, 58/59, 64-78 (in Japanese).

Kawai, K., Katayama, T. and Imabayashi, H. (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. Hiroshima Univ*., 45, 1-8.

Kawai, K., Imayoshi, Y. and Imabayashi, H. (2007): Differences in

allele frequency at ME-1 locus among four subspecies of Japanese white-spotted char *Salvelinus leucomaenis*. *Fish. Sci*., 73, 1398-1400.

Kimura, S. (1989): *Salvelinus leucomaenis* f. *imbrius*. Kawanabe H., Mizuno N. eds.: Japanese freshwater fishes, Yama To Keikoku-sha, Tokyo, 128-131 (in Japanese).

Lynch, M. (1990): The similarity index and DNA fingerprinting. *Mol. Biol. Evol*., 7, 478-484.

Miyaji, D., Kawanabe, H. and Mizuno, N. (1986): Genshoku nihon tansuigyorui zukan. Hoikusha, Osaka (in Japanese).

Nei, M. (1975): *Molecular Population Genetics and Evolution*. North-Holland, Amsterdam.

Numachi, K. (1975): *Isozyme*. In: Japanese Society of Fisheries Science ed.: Suisangaku Series 9, Biochemical Classification of Fish species. Kouseishakouseikaku, Tokyo, 49-64 (in Japanese).

Obata, H. (1991): *Geographical features of the Chugoku Region*. Kokin Shoin, Tokyo, (in Japanese).

Oshima, M. (1961): Studies on Japanese chars. *Chojushuho*, 18, 3-70 (in Japanese).

Sato, T. (1963): Gogi (*chars distributed in the Chugoku Region*). Research Reports of Cultural Assets in Hiroshima Prefecture Ⅲ. (Vol. Natural Monument). Education Commission, Hiroshima Prefecture, 3-30 (in Japanese).

Taguchi, T. (2005): *Field guide 3, Japanese fishes* (Freshwater ones), Shogakukan, Tokyo (in Japanese).

Takeshita, K. (1988): Distribution of genus *Salvelinus* in the Chugoku Region. Master thesis, Graduate School of Education, Hiroshima University, 7, 123-126(in Japanese).

Yasue, Y. (1981): *Chars in the southern limit*. Yoshimoto M., Fukui T. eds.: Iwana and Yamame, Sanposhuppan, Tokyo, 59-66 (in Japanese).

（2010 年 8 月 31 日受付）
（2010 年 11 月 19 日受理）