生物圏科学
Biosphere Sci.
**51**：1－8 (2012)

# Composition of subspecies, head spot types and genetic types of charr distributed in the Hino River, Japan

Koichiro Kawai, Hidetoshi Saito and Hiromichi Imabayashi

*Graduate School of Biosphere Science, Hiroshima University, Kagamiyama 1-4-4,*
*Higashi-hiroshima, Hiroshima 739-8528, Japan*

**Abstract** Composition of 2 subspecies of white-spotted charr, *Salvelinus leucomaenis imbrius* (“Gogi”) and *S. l. pluvius* (“Nikko-iwana”), head spot types and genetic types were investigated for the Hino River, Tottori Prefecture, Japan. A total of 38 charr samples was collected from the 19 branches. All the branches were categorized into 8 groups based on the mountain mass from which the branch originates. Nikko-iwana was distributed in the Daisen, Futago and Hanami mountain masses in the eastern part of the basin and was not distributed in the other 5 mountain masses in the western part. In contrast, Gogi was distributed in the western Onibayashi, Dogo, Mikuni, Senzu and Takairi mountain masses and was not distributed in the 3 eastern mountain masses. For head spot types, C, E, F, G and H were distributed in the 3 eastern masses whereas A, B and D were not recorded from these masses. On the other hand, all the types were distributed in the 5 western masses. For genetic types, 6 and 9 haplotypes were Nikko-iwana- and Gogi-specific, respectively. Only a haplotype comprised both subspecies. In the dendrogram, 5 haplotypes constructed an exclusive clade of Nikko-iwana whereas 6 haplotypes constructed an exclusive clade of Gogi. However, the remaining clade was constructed by both subspecies. These results suggest the co-existence of both subspecies in the Hino River, multiple origins of Gogi, movements of charr by topographic events and plasticity of the head spots.

**Key words:** charr, genetic relationship, Gogi, Nikko-iwana, *Salvelinus*

## INTRODUCTION

Two subspecies of the white-spotted charr, *Salvelinus leucomaenis* (Pallas) (called “Iwana”), including *S. l. leucomenis pluvius* (Hilgendorf) (called “Nikko-iwana”) and *S. l. imbrius* (Jordan et McGregor) (called “Gogi”), are distributed in the rivers originating from the Chugoku Mountain Chains, Japan (Hosoya, 2000). The taxonomic status of the 2 subspecies is still controversial (Oshima, 1961; Inamura & Nakamura, 1962; Imanishi, 1967; Miyaji *et al.*, 1986; Kimura, 1989). Gogi is distinguished from Nikko-iwana by clear white spots on the dorsal surface of its snout (Miyaji *et al.*, 1986; Hosoya, 2000).

For the Sea of Japan side, Nikko-iwana is distributed in the rivers westernmost to the Hino River, Tottori Prefecture (Kimura, 1989). However, the distribution limit of Gogi is also still controversial. A Gogi-like charr has been described in the Yata River, Hyogo Prefecture (Anonymous, 1974). According to Kimura (1989), Gogi is distributed in the rivers easternmost to the Hii River, Shimane Prefecture. In the previous study, we have reported the co-existence of both subspecies in the Hino River Basin (Kawai *et al.*, 2011).

---

Accepted on September 4, 2012

In the present study, we focused on the charr populations of the Hino River, Tottori Prefecture, and categorized the whole basin into several mountain masses from which multiple branches originate, in view of range expansion of charr by taking advantage of topographic events. By the examination of the distribution pattern of 2 subspecies, head spot type and genetic type in the branches of the Hino River, the meanings of the co-existence of 2 subspecies in this basin were discussed.

## MATERIALS AND METHODS

We tried to collect charr samples from as many branches of the Hino River as possible (Fig. 1). We performed sampling at the highest possible upper reaches for collection of native fish only using earthworm as a main bait. Samples were transported alive to the laboratory using a potable aeration system with gentle cooling. Samples were identified by the description in Hosoya (2000). Additionally, fish with remarkably worn fins or completely white belly, which are general features of cultured or introduced fish, were excluded. After killing by bleeding, we measured the body sizes of the samples, dissected the liver and stored it in an Eppendorf tube at -20 ℃ until analysis.

Fig. 1. Map of the 19 branches of the Hino River Basin. Eight mountain masses are also shown.

Fish is photographed in the dorsal view by a digital camera (E-510, OLYMPUS, Tokyo, Japan) and head spots were observed and typed according to the method in Kawai *et al.*(2000)

Template DNA was prepared from the samples using DNeasy Tissue Kit (Quiagen, 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 ℃ for 11 min, followed by 30 cycles of denaturation at 94 ℃ for 30 s → annealing at 55 ℃ for 30 s → extension at 72 ℃ for 1 min and a final extension at 72 ℃ for 7 min.

Sequencing was performed directly with the Genetic Analyzer 3130xl (Applied Biosystem, CA, USA). Multiple alignment was performed with ClustalW software (Thompson *et al.*, 1994).

A dendrogram was constructed by the maximum parsimony method using PAUP 4.0 (Swofford, 2000).

## RESULTS

A total of 38 fish samples were collected from 19 branches. All the branches were categorized into 8 groups based on the mountain mass from which the branch originates (Table 1). Total and body lengths ranged from 12.4-24.9 and 10.2-22.3 cm, respectively. Body weights were in the range of 15.2-134 g.

Table 1 Categorization of 19 branches by mountain mass.

| Branch | Mountain mass | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Daisen | Futago | Hanami | Onibayashi | Dogo | Mikuni | Senzu | Takairi |
| Fukatani | | | | | | | ○ | |
| Funatani | ○ | | | | | | | |
| Haitani | | | | | | | ○ | |
| Hagiyama | | | | | | | ○ | |
| Hosshoji | | | | | | | | ○ |
| Iwami | | | ○ | | | | | |
| Kitani | | | | | | | ○ | |
| Koebi | ○ | | | | | | | |
| Kogumai | | | | ○ | | | | |
| Kuzuka | | | | | ○ | | | |
| Miyamaguchi | ○ | | | | | | | |
| nameless | | | | ○ | | | | |
| Niiyama | | | | | | ○ | | |
| Obara | | | | | | | ○ | |
| Oe | ○ | | | | | | | |
| Sakago | | | | | | | ○ | |
| Sakaibara | | ○ | | | | | | |
| Shirami | ○ | | | | | | | |
| Tannaka | | | | | | | ○ | |
| Yato | | | | | | | ○ | |
| Yukawa | | | | | ○ | | | |

Table 2 Distributions of 2 subspecies, head spot types and haplotypes in the 8 mountain masses in the Hino River Basin.

| Mt. Daisen | Nikkoiwana | | | | | | | | Gogi | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spot type | A | B | C | D | E | F | G | H | A | B | C | D | E | F | G | H |
| Haplotype | | | | | 5,5,5,6 | 2,5,5,5,5,15 | | | | | | | | | | |

| Mt. Futago | Nikkoiwana | | | | | | | | Gogi | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spot type | A | B | C | D | E | F | G | H | A | B | C | D | E | F | G | H |
| Haplotype | | | 4 | | | 1 | | 4 | | | | | | | | |

| Mt. Hanami | Nikkoiwana | | | | | | | | Gogi | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spot type | A | B | C | D | E | F | G | H | A | B | C | D | E | F | G | H |
| Haplotype | | | | | 3 | 3 | 1 | | | | | | | | | |

| Mt. Onibayashi | Nikkoiwana | | | | | | | | Gogi | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spot type | A | B | C | D | E | F | G | H | A | B | C | D | E | F | G | H |
| Haplotype | | | | | | | | | 16 | 15 | | | 8 | | 9 | |

| Mt. Dogo | Nikkoiwana | | | | | | | | Gogi | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spot type | A | B | C | D | E | F | G | H | A | B | C | D | E | F | G | H |
| Haplotype | | | | | | | | | | 9 | 7 | | | 7 | | |

| Mt. Mikuni | Nikkoiwana | | | | | | | | Gogi | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spot type | A | B | C | D | E | F | G | H | A | B | C | D | E | F | G | H |
| Haplotype | | | | | | | | | | | 10 | | | | | |

| Mt. Senzu | Nikkoiwana | | | | | | | | Gogi | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spot type | A | B | C | D | E | F | G | H | A | B | C | D | E | F | G | H |
| Haplotype | | | | | | | | | 14,16 | 12 | 9,9,11 | | 11,13 | 9,16 | | 9 |

| Mt. Takairi | Nikkoiwana | | | | | | | | Gogi | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spot type | A | B | C | D | E | F | G | H | A | B | C | D | E | F | G | H |
| Haplotype | | | | | | | | | 9 | 9 | | 9 | | | | |

## 1. Distribution of the 2 subspecies

Nikko-iwana was distributed in the Daisen, Futago and Hanami mountain masses and was not distributed in the other 5 mountain masses. In contrast, Gogi was distributed in the Onibayashi, Dogo, Mikuni, Senzu and Takairi mountain masses and was not distributed in the other 3 mountain masses (Table 2).

## 2. Distribution of head spot types

Head spot types E and F; E, F and G; and C, F and H were distributed in the masses Daisen, Hanami and Futago, respectively. All the types excepting D and G were distributed in the Senzu Mountain Mass. Types A, B, E and G; B, C and F; and A, B and D were distributed in the masses Onibayashi, Dogo and Takairi, respectively. Only the type C was distributed in the Mikuni Mountain Mass (Table 2).

## 3. Haplotype distribution

A 568 bp region in the alignment of the mitochondrial cytochrome *b* genes was sequenced. A total of 23 nucleotide positions were polymorphic, and 16 haplotypes were identified.

Haplotypes 1-6 comprized only Nikko-iwana whereas haplotypes 7-14 and 16 comprized only Gogi. Only a haplotype 15 comprised both subspecies.

Haplotypes 2, 5, 6 and 15; 1 and 4; and 1 and 3 were distributed in the masses Daisen, Hanami and Futago, respectively. Haplotypes 9, 11, 12, 13, 14 and 16; 8, 9, 15 and 16; 7 and 9; were distributed in the Senzu, Onibayashi and Dogo Mountain Masses, respectively. Only haployypes 9 and 10 were distributed in the Takairi and Mikuni Mountain Masses, respectively (Table 1).

### 4. Dendrogram

Haplotypes 1, 2, 3, 4 and 6 constructed an exclusive clade of Nikko-iwana whereas haplotypes 8-13 constructed an exclusive clade of Gogi. However, the remaining clade was constructed by both subspecies (Fig. 2).

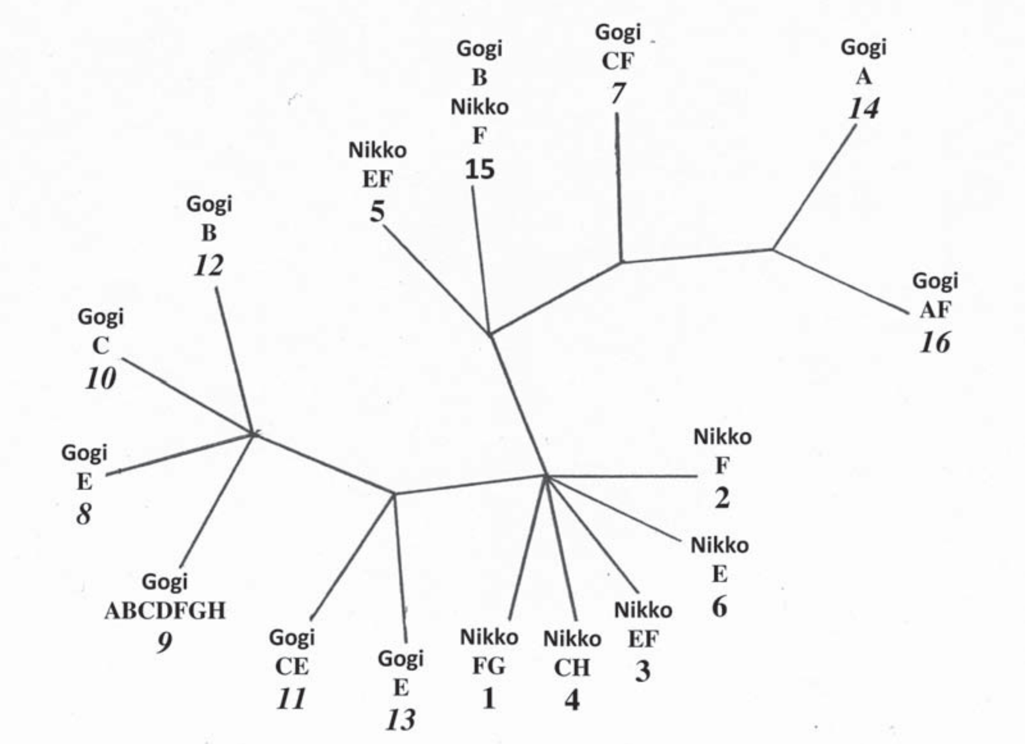

Fig. 2. A dendrogram of 16 haplotypes of Nikko-iwana and Gogi based on cytochrome *b* using PAUP. **1-6**, Nikko-iwana-specific; ***7-14, 16,*** Gogi-specific; **15**, common to Nikko-iwana and Gogi.

## DISCUSSION

In this study, co-existence of 2 subspecies of charr, Nikko-iwana and Gogi, in the Hino River Basin was confirmed and investigated in relation to head spot type and genetic type. Furthermore, Nikko-iwana was proven to be distributed only in the eastern part of the basin whereas Gogi distributed only in the western part. However, Matsui (2012) reported the collection of Gogi-like charrs in the Daisen Mountain Mass and even in the Sendai River Basin, situated in the easternmost region of Tottori Prefecture. Yasue (1981) also described on the distribution of Gogi-like charr in the Tenjin River Basin, situated in the middle of Tottori Prefecture. In the preliminary study, we also collected Gogi-like charr in the Katsuta River, originating from the northern slope of Mt. Daisen. Thus, the following scenario seems to be likely. Gogi had expanded the range eastwards in the Chugoku Mountains at the first. Later, Nikko-iwana had succesfully expanded the range westwards in this region and replaced Gogi. In that period, however, orogeny of the Daisen Mountain Mass became active (Obata, 1991) and thereby the expansion of Nikko-iwana ceased. Indeed, Gogi was estimated to have been derived earlier than Nikko-iwana (Numachi, 1975).

In this study, haplotypes 8-13 constructed an exclusive clade of Gogi, whereas haplotypes 5, 7, 14, 15 and 16 constructed a distinct clade with both subspecies. This means a possibility of multiple origins of Gogi. Thus, Gogi may comprise a derived group from Nikko-iwana that expanded seaward and southwards in the glacial periods (Yamamoto *et al.*, 2004) and were selected for fitness in the stream-like environments of the western Chugoku Mountains, resulting in clear, short and wide spots on the snout (Kawai et al, 2000), and another group that invaded into the Honshu Island via the Korean Peninsula and subsequently expanded the range eastwards, similar to that of the cyprinid fishes (Mizuno, 1987) in the Pleistocene. The presence of haplotype 15, comprising both subspecies, may suggest this possibility.

In this study, haplotype 5 was the most dominant in Nikko-iwana and distributed only in the Daisen Mountain mass. On the other hand, haplotype 9 was the most dominant in Gogi and distributed only in the western mountain masses. This means a possibility of some movements of charr by taking advantage of highland marshes appearing at flood or stream capture events frequently occurring in peneplain-like topography of the western Chugoku Mountains (Obata, 1991).

Many haplotypes comprised multiple head spot types. Haplotype 9 consisted of all the spot types excepting type E. This means a high level of morphological as well as ecological plasticity of charr by adaptation to the environments (Inamura, 1981). However, the lack of the spot types A, B and D in Nikko-iwana might imply its inability to have large, wide and short spots on the snout, resulting into a low fitness in such peneplain-like environments.

Native populations of both Nikko-iwana and Gogi have gradually but certainly diminished throughout the Japan (Sato, 1998) due to various environmental changes, the increase of fishermans and a large scale of stocking of competitive species such as *Oncorhynchus masou masou* (Brevoort) and *O. masou ishikawae* Jordan et McGregor. Particularly, some small local populations seem to be highly valuable biologically, e.g., Gogi distributed in the Kando River, Shimane Pref. and Nikko-iwana in the Arakawa River, Saitama Pref. Systematic studies by all the researchers in Japan should be conducted without any conflicts and egos before their extinction throughout the Japanese mountain rivers.

## REFERENCES

Anonymous. 1974. River fish. In: Hyogotanken Shizen-hen, (ed. by Kobeshinbun), Kobeshinbunsha, Kobe: 339-343 (in Japanese).

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

Hosoya, K. 2000. Salmonidae. In: Nihonsan gyorui kensaku (ed. by Nakabo, T.), 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)

Inamura, A. 1981. Variation and phylogeny of Japanese *Salvelinus*. In: Special on freshwater fishes Salvelinus (ed. by Kimura, E.), Preservation Association of Freshwater Fishes, Osaka: 14-18 (in Japanese).

Inoue, J. G., Miya, M., Tsukamoto, K. and Nishida, M. 2000. Complete mitochondrial DNA sequence of the Japaenese sardine *Sardinops melanostictus*. *Fish. Sci.*, **66**: 924-932.

Kawai, K., Masuhara, A. and Imabayashi, H. 2000. Relationships between the spot patterns on the head of the Japanese char *Salvelinus leucomaenis*, distributed in the Chugoku Region, and water quality. *Fish. Sci.*, **66**: 901-907.

Kawai, K., Inoue, T., Saito, H. and Imabayashi, H. 2011. Distribution of two subspecies of white-spotted charr, *Salvelinus leucomaenis imbrius* and *S. l. pluviu*s, in the rivers flowing into the Sea of Japan, and their genetic relationships, based on mitochondrial DNA sequence. *Bull. Hiroshima Univ. Mus*. **3**: 23-30.

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

Matsui, S. 2012. Gogi, fishing in the rock sides. In: Fly Fisher (ed. by Yagi, K. ), Tsuribitosha, Tokyo, **225**: 40-45 (in Japanese)

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

Mizuno, N. 1987. Establish of Japanese fresh water fish fauna. In: Japanese freshwater fish – its distribution, variation and speciation – (ed. by Mizuno, N. and Goto, A.), Tokai University Press, Tokyo: pp. 232-244 (in Japanese).

Numachi, K. 1975. Isozyme. In: Suisangaku Series 9, Biochemical Classification of Fish species (ed. by Japanese Society of Fisheries Science), Koseishakoseikaku, Tokyo: pp. 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, S. 1998. *Setogiwa no Sakanatachi*, Tsuribitosha, Tokyo (in Japanese).

Swofford, D. L. 2000. *PAUP: Phylogenetic analysis using parsimony*. Version 4. Sinauer Associates Sunderland.

Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the Sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucreic Acids Res.* **22**: 4673-4680.

Yasue, Y. 1981. The charr in the southern limits. In: Iwana and Yamame (ed. by M. Yoshimoto), Sanpo Press, Tokyo: 60-66 (in Japanese)

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

# 日野川水系に分布するイワナ属魚類の亜種，頭部斑紋型，遺伝子型の組成について

河合幸一郎・斎藤英俊・今林博道

広島大学大学院生物圏科学研究科　〒739-8528　東広島市鏡山1-4-4

**要　旨**　鳥取県日野川水系におけるイワナ属魚類について，ゴギとニッコウイワナ，頭部斑紋型，遺伝子型の組成を調べた。計19の支流から38個体のサンプルが採集され，支流はその発する山塊ごとにグループ化された。ニッコウイワナは水系東部の大山・二子・花見山塊から発する支流に分布したが西部5山塊からの支流には分布せず，対照的にゴギは水系西部の鬼林・道後・三国・船通・鷹入山塊に分布したが東部3山塊には分布しなかった。斑紋型では東部3山塊ではＣ・Ｅ・Ｆ・Ｇ・Ｈは記録されたがＡ・Ｂ・Ｄ型は記録されなかった。一方，西部5山塊ではすべての型が記録された。遺伝子型では6および9つのハプロタイプがそれぞれニッコウイワナおよびゴギ特異的であり，1タイプのみ共通であった。デンドログラムではニッコウイワナおよびゴギそれぞれ5および6つのハプロタイプで構成される亜種特異的なクレードが見られたが，もう一つのクレードは両亜種で形成された。これらの結果は，日野川における両亜種の混棲，ゴギの複数起源性，地形変動等による分布拡大，さらに斑紋の易変異性を示唆する。

**キーワード**：分布，ゴギ，ニッコウイワナ，斑紋，遺伝子