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Translocation of White-Spotted Charr – Identification of Hybrid Population from its Molecular Record –

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ABSTRACT:
The charr population on the Tone River has been contaminated genetically with fishes released by fishery for over half a century. Although some native populations are thought to remain in upper streams around the Yunishigawa dam, there is concern that dam construction may threaten these populations with genetic contamination, because part of their habitat will be under the reservoir for flood control. To conserve the valuable population of native white-spotted charr that inhabit around the dam, a preservation area was selected based on their genetic information. Our analyses confirmed genetic contamination in two mountain streams. The body length of the contaminated individuals showed that the introduced charr genetically affects multiple generations. Only a few populations were found to have lower contamination levels than other sites. Also, the frequencies of introduced genotypes are less in the upper mountain streams than in the lower areas. Based on these results, four local populations were selected for conservation. The individuals in the upper area were transplanted to the uppermost area.

Keywords: native population, genetic contamination, introduced population, genetic structure

1. YUNISHIGAWA DAM AND WHITE-SPOTTED CHARR

The Yunishigawa-Dam, which is 119 m high and has a catchment basin of 102 km^2, is a multi-purpose dam currently being constructed on the Kinu River in the Tone River Basin. The white-spotted char, which is a landlocked species of salmonoid, breeds in the Tone River Basin, including the reservoir area of the dam. Although the species is distributed over a large part of Japan, the local populations have decreased recently. One of the major cause of the decrease is genetic disturbance. Since the 1960s, juvenile fishes of white-spotted char from other areas or hybrid species with immigrant fishes have been released throughout Japan by fisheries, including the Yunishigawa River, and individuals of native local populations have been disturbed genetically as a result. Today, they are listed in the Red Data Book Categories (Environment Agency 1997) as “insufficient data”. The fisheries along the Tone River also have released immigrant fishes, but they have not released juveniles in several mountain streams in order to keep the local population of white-spotted charr in their home area.

There are three mountain streams which seem to have escaped the effect of releasing juveniles, two of which have populations of white-spotted charr. One of the streams is divided into five areas by five weirs and one waterfall, and the other stream is divided into four areas by one weir and two waterfalls (Fig. 1). All of the individuals of each population can migrate downstream, but a few charr seem to migrate to upper areas from lower areas. Native local populations of white-spotted char seem to remain in the areas above these facilities.

The Yunishigawa Dam project, when completed, will pose the risk of genetic disturbance, since the water level of the reservoir will rise higher than the weirs for flood control that currently prevent movement of impure-blooded individuals. To preserve pure-blooded white-spotted charr, we examined the migration of pure-blooded individuals to St. 5, 7 and 8, shown in Fig.1, which are higher than the surcharge reservoir level.

In preservation programs, it is important to confirm that the populations have never been contaminated by other populations. Another threat to blood purity of individuals is the release by people for their own pleasure. In this program, a molecular method was applied to the charr population to detect historical contamination. Populations for preservation were then chosen based on the results.

2. OUTLINE OF MOLECULAR METHOD OF THE OBSERVATION PROGRAM

2.1. Mitochondrial Deoxyribonucleic Acid and Nuclear Deoxyribonucleic Acid

All creatures have genetic information in their bodies, which includes important information on building and maintaining the body. This information is recorded by only four kinds of deoxyribonucleic acid (DNA) without exception in all creatures, including viruses. In humanoids, the number of arrangements of DNA is 16, 569 base pairs (bp) on mitochondrial DNA, and 3.2 billion on nuclear DNA. Although this information is copied exactly to the next generation, occasionally fortuitous mistakes occur, and are passed on to the next generation. These fortuitous mistakes have accumulated in DNA, and convey individual information such as differences in the arrangement of DNA of specific parts or differences in the length of specific parts. A suitable analysis method will be chosen from among the many methods based on cost, target population, requirement for reliability of data, and volume of sample.

Mitochondrial DNA is a highly preserved area which has a lower frequency of genetic variation compared with nuclear DNA. These genetic variations are often used for comparison among species. The genetic variations of mitochondrial DNA of various creatures can be retrieved for free from databases such as DNA Databank of Japan (DDBJ; http://www. ddbj.nig.ac.jp/) and Fishbase (http://www.fishbase.org).

Although mitochondrial DNA is used for comparison among species, it is not sufficient to detect impure-blooded populations. All of the genetic information of mitochondrial DNA is passed by the female to the child every other generation without gene recombination. The mitochondrial DNA method cannot detect past contamination by immigrant male fishes. Although a male cannot pass on his mitochondrial DNA, he passes on half of his nuclear DNA to all of his children, and complete Y-chromosome DNA to his sons. Nuclear DNA is more variable than mitochondrial DNA and gives more information for population analysis.

The method of microsatellites, which are repeating sequences of 2-6 base pairs of DNA, is one way to analyze nuclear DNA. It is sensitive and gives high reproducibility.

In this study, mitochondrial DNA analysis was used to ensure introgression and microsatellite analysis was used to estimate the historical introgression.

2.2. Sampling and DNA Extraction

Fin samples from 192 charr were collected from three parts of the Yunishigawa River and eight areas of branch rivers in 2008 (Fig. 1). After removal of fin tissue, all of the charr were returned to their original habitat. Samples were preserved in 95% ethyl alcohol until DNA extraction using a phenol-chloroform extraction protocol after proteolysis by Proteinase K (Qiagen, Germany).

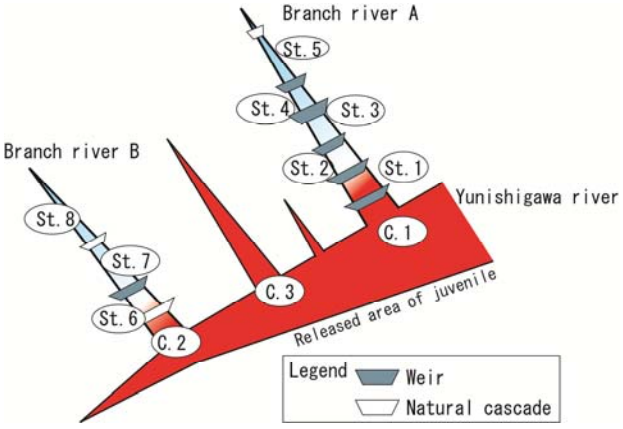

Figure 1. Location of each site

3. RESULT

3.1. Mitochondrial Deoxyribonucleic Acid Analysis

Kubota, et al. found several types of mitochondrial DNA of charr in the Tone River population, of which a 557-bp arrangement was compared in cytochrome b on mitochondrial DNA which is shown on GEDIMAP (GEnetic DIversity and its DIstribution Map; http://gedimap.zool.kyoto-u.ac.jp).

DNA arrangements of mitochondrial DNA of each sample were analyzed by ABI Prism 3100, and compared to the given arrangement for outline search of

Table 1. Detected types of mitochondrial DNA at each station

River	Station	Type of Mitochondria DNA
Branch river A	S1	N2 (6), N1 (1), A1 (4)
	S2	N1 (14)
	S3	N1 (11)
	S4	N1 (17)
	S5	N1 (20)
Branch river B	S6	N1 (13)
	S7	N1 (20)
	S8	N1 (20)
Yunishigawa River	C1	N1 (13), N2 (5), A1 (3)
	C2	N1 (29), N2 (2), A2 (1), B (1)

N1, N2: Origin in Tone River Basin
A1, 2, 3 and B: never reported in Tone River Basin.
Numbers in parentheses show the number of individuals.

immigrated individuals. Some types have been reported in the Tone River Basin, but several types have been reported in other basins, thus verifying contamination with immigrant fishes (Table 1).

3.2. Microsatellite Analysis

Nine microsatellite markers of Japanese charr have been developed by Kubota et al. To estimate the genetic differentiation between the populations at each site and between different generations, the genetic diversity and population genetic structure were estimated using *Structure (Ver. 2.3.1)*. The program *structure* implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers (http://pritch.bsd.uchicago.edu/structure.html).

All of the microsatellite data except one misanalysis individual data were clustered to the four assumed populations. The results for the gene structure of each individual are distinguished by bars of four different colors (Fig. 2). The gene structure of two micro local populations in each branch river, which were isolated from the Yunishigawa River by a cascade or weir, shows specific differences among each other (genetic element colored red and green). It was assumed that these two genetic elements originate in the native population of this area.

The genetic elements colored yellow or red are assumed to originate in a contaminated population of the Yunishigawa River, for which genetic contamination was verified by mitochondrial data. The yellow element of the contaminated population confirmed the majority at St. 2, although no impure-blooded individuals were confirmed by mitochondrial DNA. Furthermore, this element was confirmed for several individuals at Stations 3, 4 and 5. These results show that there are few pure-blooded individuals in branch river A. The red element was also confirmed slightly for some individuals in both branch rivers. The body length of contaminated individuals in each branch river is distributed over several generations of charr. From these results, we conclude that genetic contamination of white-spotted charr has occurred widely and over a long term.

3.3. Preservation Plan

Gene analysis shows that no pure-blooded population remains in these areas. However, the occurrence of elements of the introduced charr is low at Station 5 in branch river A and Stations 6, 7, 8 in branch river B. To preserve these low-contaminated populations of white-spotted charr, preservation planning is being done before dam construction.

Station 5 of branch river A is the uppermost area of the habitat for white-spotted charr in branch river A, and is higher than the reservoir even when flooded. Thus, there is no concern of invasion by impure-blooded charr. No individuals have been translocated to other areas, and no modifications are being done at Station 5 to sustain the present conditions.

Stations 6 and 7 of branch river B will be threatened by new contamination with impure-blooded charr in downstream areas after dam construction. Station 8 is the only safe habitat for low-contaminated populations in branch river B in the future. To increase genetic diversity in Station 8, all individuals at Stations 6 and 7 were translocated to Station 8.

4. CONCLUSION

In this study, genetic analysis enabled accurate planning for preserving white-spotted char. There are local populations in other species, especially fresh water species, and they often pose a similar threat of contamination with introduced species. Sometimes, weirs and dams protect these species, and restoration of physically continuous routes by fishway or restoration of habitat may help to attract these species. Gene analysis will be used for protecting native ecosystem integrity in the future.

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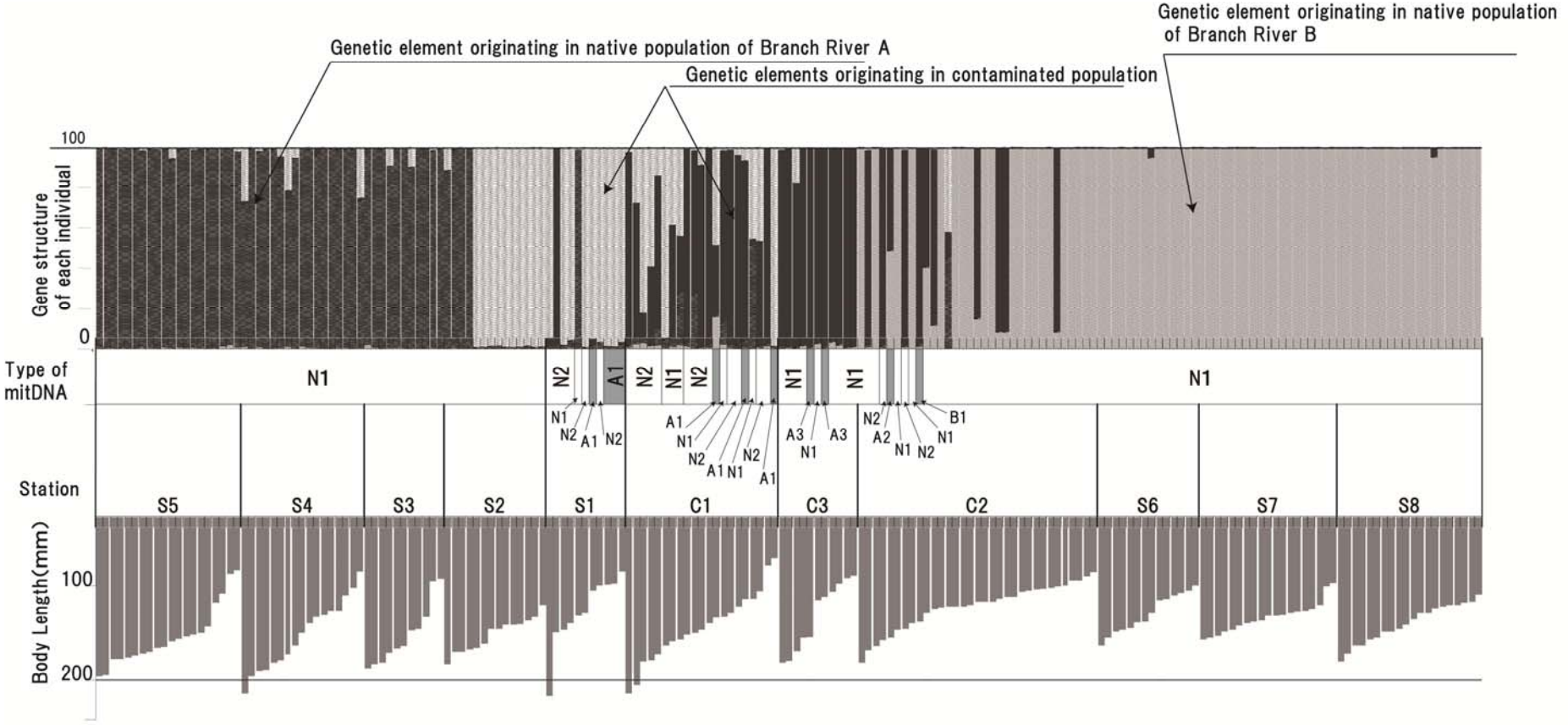

Figure 2. Gene structure, type of mitochondrial DNA and body length of each individual