Genetic Differentiation between Two Types of Dark Chub, 
*Zacco temmincki*, in Japan

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Abstract Two types of the dark chub, *Zacco temmincki*, collected from 10 river systems in Japan were genetically characterized at 27 protein coding loci using starch-gel electrophoresis. They were fixed for different alleles at 13 loci. No hybrid individuals were observed, even in specimens collected in stations where both types appear sympatrically, indicating that each type of the dark chub represents a distinct species.

The dark chub, *Zacco temmincki*, is widely distributed from Korea to the western parts of Japan (Uchida, 1939; Bănărescu, 1968; Nakamura, 1969). We recognized two different types, here called Type A and Type B, in Japanese populations based on some morphological differences (Watanabe and Mizuguchi, 1988). The fish of Type A possesses a transparent dorsal fin with a faint red blotch and their front edge of the pectoral fin is red, whereas the dorsal fin of Type B is dark yellow. The number of pored scales of Type A tends to be larger than that of Type B. Type B is distributed more widely in the western part of Japan than Type A, which extends from Tokai, through Lake Biwa to the Sanyo District along the Seto Inland Sea. The overall abundance of Type A is far less than that of Type B.

We have examined morphological, genetic and taxonomic aspects of these two types. This paper deals with the biochemical population genetics of 17 populations of the dark chub collected from 10 river systems in Japan. The purpose of the present study is to define the magnitude of genetic difference between the two types. Allozyme analysis suggested that the dark chub distributed in the Korean Peninsula also comprises two distinct species, the so-called MM and MS types (Yang and Min, 1987). The purpose of the present study also includes suggestion of a relationship between Japanese and Korean dark chub populations.

Materials and methods

Samples were collected from 10 river systems in middle and western areas of Honshu, Japan, by hand net and trap bottle during the period from 1988 to 1990. Sample locations are shown in Fig. 1 and sample size is listed in Table 2. Whole fish bodies were frozen immediately following collection until processed for electrophoresis. Horizontal starch-gel protocols followed May et al. (1979) and Harris and Hopkinson (1976). An initial identification between Types A and B was made based on the difference in color patterns of the dorsal and pectoral fins.

A list of enzyme stains used, their abbreviations, Enzyme Commission number, the numbers of loci expressed and others are given in Table 1. Tissues of the liver, skeletal muscle, eye ball and heart were used. Locus and gene nomenclatures follow Allendorf and Utter (1979) and Shaklee et al. (1990). If multiple loci coded for a protein, the loci were numbered starting with the one which encoded the protein with the least anodal migration. The most common allele at a locus of the B type was designated *100*, and other alleles were assigned numbers according to the mobility of their homomeric protein products relative to that of the *100* allele.

Homology of the loci which showed different mobilities on the gels for each type and were considered to be fixed for different alleles were confirmed.
Fig. 1. Map of Japan and the Korean Peninsula showing the sites where dark chub were sampled (○; Type A, ●; Type B, ◦; Types A and B) and its endemic range (shaded; Uchida, 1939, Bănărescu, 1968; Nakamura, 1969).

1; Shinbori River, 2; Nagara R., 3; Ibi R., 4; Suzuka R., 5; Ano R., 6; Kumozu R., 7; Tokida R., 8; Ane R., 9, 10; Yasu R., 11; Asahi R., 1; Ise Bay, II; Seto Inland Sea. ——; Ibuki Mountains, ———; Suzuka Mountains, ————; Nunobiki Mountains.

by breeding tests. For the remaining loci, the Mendelian nature of the electrophoretic variants was inferred from the banding patterns on the gels according to the following criteria: (1) Banding patterns had to be consistent with the known molecular structure of that protein. (2) When a gene was expressed in more than one tissue, variant phenotypes had to be parallel among tissues (Grant et al., 1983).

Results

Among 27 presumptive loci surveyed, 7 loci were polymorphic in the Japanese dark chub collections following the criterion that a locus is considered polymorphic in populations in which the frequency of the most common allele is less than 0.99.

At 13 loci, Type A was fixed for different alleles from those of Type B (Fig. 2). Allelic frequencies of 17 dark chub populations collected from Japanese rivers are presented in Table 2. At all of the polymorphic loci, the deviation of observed number from the expected number was not significant (assuming Hardy-Weinberg equilibrium). Nei's (1978) genetic distance value \(D\) (revised by sample size) using 27 loci was 0.64 between Types A and B when the total collections were combined.

In Type B, the \(PEPA^*\) locus was almost fixed for different alleles for the east and west collections divided at the Ibuki, Suzuka and Nunobiki Mountains. Similarly, the \(PEPC^*\) locus of Type A was almost fixed for different alleles for each collections. Allelic variation at the \(AK^*\) locus was observed exclusively in Type B from the Ano and Kumozu River populations.

Discussion

According to our observations, Type A tends to be distributed in the lower reaches and more placid waters compared to Type B. In some stations, however, both types are distributed sympatrically and sometimes they were caught together in a single trap bottle. The lack of hybrid specimens showing