
Genetic Relationships between Japanese and Korean Odontobutis obscura
(Pisces, Odontobutidae)

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Abstract. A population of Odontobutis obscura from Goje Island, Korea, was compared genetically with Japanese populations, utilizing 18 allozyme markers. The former was closest (D=0.184±0.003) to the East Seto group, one of the four geographic groups of Japanese O. obscura, being included in the cluster of East and West Seto groups. Its divergence is likely to have occurred concurrently with the divergence of the two latter groups or their divergence together with the West Kyushu group.

Key words: Odontobutidae, freshwater goby, allozyme, Tsushima Strait.

Introduction

The donko, Odontobutis obscura (Temminck & Schlegel, 1845) (Odontobutidae), had been thought to be absent in Korea, although distributed in both Japan and China (Sakai et al., 1998), until a solitary population was found on Goje Island, Korea, facing the Tsushima Strait (Chae, 1999). Because the species is absent on Tsushima Island (Tomoda, 1970), located in the middle of the Tsushima Strait between the Korean Peninsula and Japan proper, the Goje population represents a significantly isolated colony of this species.

Japanese donko clearly differ genetically from Chinese forms according to mitochondrial DNA (mtDNA) analyses (Kobayashi et al., unpublished data). Sakai et al. (1998) considered that Japanese donko comprised five geographic groups, differing in their allozyme allelic composition, namely the San-in-Biwa-Ise, East Seto, West Seto, West Kyushu and Hikimi groups: The last, having a unique cephalic lateral line character, has been recently described as a new species, O. hikimius Iwata and Sakai (2002).

The establishing of an affinity of the Korean population to a particular geographic group of Japanese populations may throw some light on the questions of freshwater fish exchange between the Japanese Archipelago and the Korean Peninsula in the glacial periods. Accordingly, we compared allozyme allelic compositions and examined the relationships between Korean and Japanese populations of O. obscura, with O. hikimius as an outgroup.

Because Chae (1999) provided only a brief account of the cephalic sensory system in his morphological description of O. obscura from Goje
Island, more detailed figures and notes on the cephalic sensory organs are included here.

**Materials and Methods**

Five specimens of *Odontobutis obscura* for allozyme analysis, NSMT-P 61274 (National Science Museum, Tokyo), 40.7–94.0 mm SL, and three specimens for observation of cephalic sensory papillae, NSMT-P 61754, 53.6–108.0 mm SL, were collected from the Sanyang River (length about 12 km) at about 5.5 km from the river mouth (above Guchon Reservoir), Goje Island, Korea (Fig. 1) on April 7, 2001. No other Korean congener (*O. platycephala* and *O. interrupta*) are known from the island.

The same 12 enzymes and 18 loci (EC numbers and loci) as in Sakai et al. (1998) were examined: aspartate aminotransferase (EC 2.6.1.1; AAT-1*), alcohol dehydrogenase (EC 1.1.1.1; ADH*), creatine kinase (EC 2.7.3.2; CK*), glycerol-3-phosphate dehydrogenase (EC 1.1.1.49; G3PDH*), glucose-6-phosphate dehydrogenase (EC 5.3.1.9, GPI-1*, GPI-2*), isocitrate dehydrogenase (EC 1.1.1.42; IDH-1*, IDH-2*), lactate dehydrogenase (EC 1.1.1.27; LDH-1*, LDH-2*, LDH-3*), malate dehydrogenase (EC

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**Fig. 1.** Sampling localities of Korean and Japanese *Odontobutis obscura* and *O. hikimius*: Guchon River population, Goje Island (GOJE), Korea (solid star); Kanakusari (KANK), Sana (SANAN), Kuzuryu (KUZR), Hii (HII) and Hamada (HAMD) River populations from San-in-Biwa-Ise group (solid circles); Kiita (KITA), Takeda (Yura River basin, TAKD), Maruyama (MARRY), Ibo (IBO) and Kurosie (KURS) River populations from East Seto group (open squares); Shinjo (SHNJ), Iyoki (IYOK), Shimanto (SMNT), Souro (SOUR) and Kunichika (KNCK) River populations from West Seto group (solid squares); Tataru (TATR), Chikugo (CHKG), Kaze (KAZE), Fukue (FKUE) and Kuma (KUMA) River populations from West Kyushu groups (open circles); *O. hikimius* from the Hikimi River (HIKM in Sakai et al., 1998) (open star).
Table 1. Alleloyme allelic compositions of 18 loci in 5 individuals of Korean *Odontobutis obscura*, allelic nomenclature following that of Sakai *et al.* (1998).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Locus</th>
<th>Allele</th>
<th>Locus</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAT-1</td>
<td><em>100</em></td>
<td>CK</td>
<td><em>100</em></td>
<td>ADH</td>
<td><em>100</em></td>
</tr>
<tr>
<td>G3PDH</td>
<td><em>100</em></td>
<td>GPI-1</td>
<td><em>100</em></td>
<td>GPI-2</td>
<td><em>100</em></td>
</tr>
<tr>
<td>IDHP-1</td>
<td><em>100</em></td>
<td>IDHP-2</td>
<td><em>100</em></td>
<td>IDH-1</td>
<td><em>100</em></td>
</tr>
<tr>
<td>LDH-2</td>
<td><em>100</em></td>
<td>LDH-3</td>
<td><em>100</em></td>
<td>MDH-1</td>
<td><em>60</em></td>
</tr>
<tr>
<td>MDH-2</td>
<td><em>130</em></td>
<td>MDH-3</td>
<td><em>100</em></td>
<td>PGDH</td>
<td><em>100</em></td>
</tr>
<tr>
<td>PGM-2</td>
<td><em>107</em></td>
<td>SOD</td>
<td><em>42</em></td>
<td>XDH</td>
<td><em>100</em></td>
</tr>
</tbody>
</table>

1.1.1.37; MDH-1<sup>*</sup>, MDH-2<sup>*</sup>, MDH-3<sup>*</sup>, phosphogluconate dehydrogenase (EC 1.1.1.44; PGDH<sup>*</sup>), phosphoglucomutase (EC 5.4.2.2; PGM<sup>*</sup>-2), superoxide dismutase (EC 1.15.1.1; SOD<sup>*</sup>) and xanthine dehydrogenase (EC 1.1.1.204; XDH<sup>*</sup>).

The data obtained from the Korean population (Goje) were compared with those of 20 Japanese representative populations from four geographic groups of *O. obscura* and *O. hikimius* (Sakai *et al.*, 1998) (Fig. 1). For tree construction, the UPGMA method (Sneath and Sokal, 1973) based on Nei’s (1972) genetic distance (*D*) was employed so as to establish the genetic relationships among them. Support for the clusters was examined by 500 bootstrap replicates, although only low values could be expected owing to the small number of examined loci.

Results and Discussion

The Goje population of *Odontobutis obscura* was monomorphic in all the loci examined (Table 1), all the alleles except for allele *60 in locus MDH-1* being shared with at least one Japanese population (see Sakai *et al.*, 1998). However, the Goje population was closest to the East Seto group of Japanese donko (*D*=0.184, Table 2). In the UPGMA dendrogram (Fig. 2), it fell into the East-West Seto cluster, being connected with the East Seto group following the KNCK population of the West Seto group (KNCK is genetically intermediate between the East and West Seto groups, Sakai *et al.*, 1998).

Nei’s *D* value between the Goje and East Seto groups corresponded to a divergence time of about 0.92, 2.3 or 3.6 million years (Myr) according to Nei’s (1975) traditional (*D*=5 Myr), Nishida’s

Table 2. Mean genetic distances (Nei, 1972) with standard deviations between pairs of Korean *Odontobutis obscura*, four geographic groups comprising five populations of Japanese *O. obscura* and *O. hikimius* (lower diagonal), and intra-group values of Japanese *O. obscura* groups (on diagonal).

<table>
<thead>
<tr>
<th>O. obscura</th>
<th>Korea</th>
<th>San-in-Biwa-Ise g.</th>
<th>East Seto g.</th>
<th>West Seto g.</th>
<th>West Kyushu g.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. obscura</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBI g.</td>
<td>0.338±0.067</td>
<td>0.174±0.048</td>
<td>0.060±0.050</td>
<td>0.096±0.047</td>
<td>0.262±0.058</td>
</tr>
<tr>
<td>ES g.</td>
<td>0.184±0.003</td>
<td>0.303±0.071</td>
<td></td>
<td>0.319±0.048</td>
<td>0.122±0.067</td>
</tr>
<tr>
<td>WS g.</td>
<td>0.372±0.059</td>
<td>0.305±0.048</td>
<td></td>
<td>0.460±0.043</td>
<td></td>
</tr>
<tr>
<td>WK g.</td>
<td>0.262±0.082</td>
<td>0.363±0.072</td>
<td></td>
<td>0.520±0.034</td>
<td></td>
</tr>
<tr>
<td><em>O. hikimius</em></td>
<td>0.493</td>
<td>0.325±0.010</td>
<td>0.460±0.043</td>
<td>0.556±0.054</td>
<td></td>
</tr>
</tbody>
</table>

g.: group.
Fig. 2. UPGMA dendrogram of Nei's (1972) genetic distance between pairs of 21 populations of Korean and Japanese *Odontobutis obscura* and *O. hikimius*. Abbreviations and symbols correspond to those in Figure 1. Bootstrap probabilities (%) for 500 replicates indicated at nodes.

(1997) Tanganyikan cichlid (*D* = 12.5 Myr) or Doadrio & Carmona's (1998) Greek cyprinid (*D* = 19.3 Myr) calibrations, respectively. While it is presently difficult to determine which estimation should be most reasonably applied to the present case, all of those dates are much earlier than the last land connection between Japan and the Korean Peninsula in the last glacial epoch (about 0.02 Myr ago, Park *et al.*, 1996).

A possible explanation is as follows: *O. obscura* extended its range to Goje Island prior to recent glacial epochs, but was prevented from dispersing beyond that island by the Korean congener, *O. platycephala* and *O. interrupta*, even though donko's allozyme evolutionary rate might have been more rapid than other freshwater fishes (as possible in many Japanese populations) (Sakai *et al.*, 1998; Matsubara *et al.*, 2001).

The bootstrap probabilities connecting the Goje, West and East Seto groups were not so high (Fig. 2).
The Goje population did not have allele *107 of PGM-2* in common with the West Seto group, although it did so with the West Kyushu group (see Sakai et al., 1998), and there exist also a hidden bootstrap probability (10%) of connecting the Goje population with the latter. On the other hand, the Goje population and San-in-Biwa-Ise group were dichotomous for alleles of three loci, G3PDH*, MDH-I* and SOD* (see Sakai et al., 1998). Therefore, it appears that the divergence of Korean donko would have occurred at about the same time as the divergence of the East and West Seto groups or of the West Kyushu and East-West Seto groups of Japanese donko.

Any suggestion that Japanese populations originated from Korean donko can be realistically rejected because O. hakimius, a sister species of O. obscura, is distributed in Japan and also because Korean donko is connected to a relatively younger cluster of Japanese donko (Sakai et al., 1998).

A more sensitive analysis, such as mtDNA sequencing, should enable greater clarification of the genetic relationships between Korean and Japanese populations of donko. A genetic comparison between Japanese and Korean Coreoperca kawamebashi, which has a similar distribution pattern to O. obscura (Choi et al., 1984; Okazaki and Jeon, 1996), may also help in further understanding aspects of divergence and dispersal to both sides of the Tsushima Strait.

Cephalic sensory organs are illustrated in Fig. 3. The sensory papillae arrangement is the same as in the Japanese donko (Iwata et al., 1985), except that the papillae row behind the eye was not connected with the uppermost longitudinal papillae row on the cheek (both sides of two specimens and right side of the smallest specimen (NSMT-P 61754) of Korean donko), such a connection occurring in 84 out of 89 specimens of Japanese donko (Iwata, unpublished). Although ambiguous because of the small number of specimens examined, some variation in this character may exist between Korean and Japanese O. obscura.

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Literature Cited


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