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Genetic Differences of *Pungitius pungitius* and *P. sinensis* in a Small Pond of the Omono River System, Japan

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Pungitius pungitius and *P. sinensis* are distributed in the northern part of Honshu and Hokkaido Islands, Japan. No genetic differences were detected between the two species in cohabiting rivers in Hokkaido (Niwa, 1987), and only one morphological difference, number of lateral plates, was observed (Takata et al., 1984). These suggest that the two species in Hokkaido should be distinguished from each other at a lower taxonomic level of species (Takata et al., 1984). However, morphological differences between coexisting *P. pungitius* and *P. sinensis* were revealed in the Omono River of Tohoku District (Tanaka, 1982). Biochemical discriminations were performed on *P. pungitius* and *P. sinensis*, cohabiting in a pond in Hiraga of the Omono River system, by using isozymes and protein alleles detected through starch gel electrophoresis in order to clarify whether or not they are reproductively isolated and to reassess their taxonomic status.

Materials and methods

Specimens of *P. pungitius* and *P. sinensis*, which were distinguishable by their lateral plate morphology according to Ikeda (1933), were collected in April 1983 at a pond of the Omono River system in Hiraga, Akita Prefecture on Honshu, the main island of Japan. This nameless pond is small and narrow (about 100×20 m) and shallow (80 cm in depth). A narrow inlet from a spring flows into the upper end of this pond and two outlets flow out at another end and connect to a tributary of the Omono River. Filamentous algae cover the muddy bottom and thin patches of vegetation are distributed along the shore.

All specimens were captured with a dip net and immediately carried to the laboratory with dry ice in an insulated container, and stored at $-40°C$ until use for electrophoretic analysis. Thirteen loci, controlling phosphoglucomutase (PGM), phosphoglucose isomerase (PGI), 6 phosphogluconate dehydrogenase (PGD), α-glycerophosphate dehydrogenase (GPD), malate dehydrogenase (MDH), superoxide dismutase (SOD), and muscle protein (MP) were analyzed by starch gel electrophoresis. The procedures for electrophoresis, staining, assumption of loci and alleles were performed as described by Takata et al., (1987).

Results

Pungitius pungitius and *P. sinensis* were mainly collected in the areas with thin vegetation along the shore of the pond but the distribution of the two species varied. Many *P. pungitius* were caught in the lower one third of this pond. All of the 17 specimens collected in outlets of the pond belonged to *P. pungitius*. The majority of the specimens caught in the upper two thirds of this pond belonged to *P. sinensis*.

Adult females of both species had ripe eggs and adult males of each species were black, their nuptial color. Some males were observed guarding deposited eggs in their nests.

Allele frequencies for 13 loci examined in samples of the two species are compared (Table 1). The phenotypic patterns for 5 out of 13 loci were polymorphic in *P. pungitius*: *Gpd*, *Pgd*, *Pgi*-II, *Pgm*-II and *Mp*-III. Two of 13 loci were polymorphic in *P. sinensis*: *Pgi*-II and *Pgm*-II. Comparison of observed frequencies of genotype on *Pgd* in *P. pungitius* and on *Pgi*-II in *P. sinensis* with Hardy-Weinberg expectations showed no statistically significant deviations.

As for the loci *Pgm*-I, displacement of alleles was observed between *P. pungitius* and *P. sinensis*. *P. pungitius* had *Pgm*-I-a, whereas *P. sinensis* possessed *Pgm*-I-b. Significant differences in allelic frequencies on *Gpd*, *Pgd*, *Pgi*-II and *Mp*-III were also detected between the two species ($P < 0.001$). Estimate of genetic distance between the two populations (Nei, 1972) was 0.4278.

Concerning the 4 loci on which significance were observed, only two individuals which belonged morphologically to *P. pungitius* were responsible for rare alleles of this species which are common to *P. sinensis*. One of them showed heterozygous patterns on *Gpd* (bc), *Pgd* (ac), *Pgi*-II (cd), but showed homozygous pattern on *Mp*-III (bb) as *P. sinensis*; the other exhibited

heterozygous on *Pgd* (ac), *Pgi*-II (cd), but the same homozygous on *Gpd* (cc) and *Mp*-III (bb) as *P. sinensis*.

Discussion

The fact that only two individuals were responsible for all rare genotypes on 4 loci (*Gpd*, *Pgd*, *Pgi*-II and *Mp*-III) in *P. pungitius* strongly suggests that these are not rare variants of *P. pungitius* on each of the loci, but, hybrids between *P. pungitius* and *P. sinensis*. Therefore, not only *Pgm*-I but also *Gpd*, *Pgd*, *Pgi*-II and *Mp*-III are regarded as displaced loci between the two populations.

Moreover, the combinations of displaced alleles on the 4 loci of the two individuals may indicate that these are not F_1 hybrids but belong to the later filial generations, because they did not show heterozygous patterns on all displaced loci.

A marked deficiency of lack of heterozygotes between sympatric populations can be taken as evidence of reproductive isolation (Dowling and Moore, 1984; Takata et al., 1987). Therefore, these displacements of alleles between the sympatric populations of *P. pungitius* and *P. sinensis* distinctly show that they lack the ability to exchange genes with each other and thus they are in almost perfect reproductive isolation.

Appearance of a few hybrids suggests that the premating isolation is not sufficient to prevent exchange of genes between populations of *P. pungitius* and *P. sinensis* in this small pond. No F_1 hybrids were observed, but later filial generations occurred suggesting that fertile F_1 hybrids exist. The ratio of hybrids to all specimens examined was, however, less than 3%. This suggests that not only premating isolation but also hybrid breakdown of postmating isolation (Dobzhansky et al., 1977) may play a role in the reproductive isolating mechanisms, since if all hybrids were viable and fertile, more hybrids would be expected to occur.

Significant differences in the average number of dorsal spines, gill-rakers, dorsal rays, pelvic rays and caudal rays were found between *P. pungitius* and *P. sinensis* cohabiting in the stream of Hiraga located near this pond in the same river system (Tanaka, 1982). Moreover, a biased distribution pattern of both species, which was observed in some tributaries of the Omono River system (Ikeda, 1950), was also detected in this pond. Genetic distance between *P. pungitius* and *P. sinensis* (D=0.4278) in this pond is almost similar to values between congeneric species in other fishes (Taniguchi et al., 1986). According to the biological species concept (Mayr, 1963), such observations as allelic displacements, genetic distance and conspicuous morphological and ecological differences suggest that *P. pungitius* and *P. sinensis* in the Omono River should be regarded as independent species.

In Hokkaido, however, neither genetic differences (Niwa, 1987) nor morphological differences, except for the number of lateral plates (Takata et al., 1984), were confirmed between the fresh water type of *P. pungitius* (Takata et al., 1987) and *P. sinensis* in the cohabiting rivers. This suggests that both of the species in Hokkaido cannot be regarded as independent species. Based

Table 1. Allele frequencies on 13 loci of *Pungitius pungitius* and *P. sinensis*.

Locus		*P. pungitius* type (N=40)	*P. sinensis* type (N=40)	Locus		*P. pungitius* type (N=40)	*P. sinensis* type (N=40)
Mdh-I	a	1.000	1.000	*Pgm*-I	a	1.000	0.000
Mdh-II	a	1.000	1.000		b	0.000	1.000
Mdh-III	a	1.000	1.000	*Pgm*-II	c	0.987	0.987
Gpd	b	0.962	0.000		e	0.013	0.013
	c	0.038	1.000	*Sod*	c	1.000	1.000
Pgd	a	0.025	1.000	*Mp*-I	b	1.000	1.000
	c	0.737	0.000	*Mp*-II	b	1.000	1.000
	d	0.238	0.000	*Mp*-III	a	0.950	0.000
Pgi-I	b	1.000	1.000		b	0.050	1.000
Pgi-II	b	0.000	0.385				
	c	0.025	0.615				
	d	0.975	0.000				

on morphological studies, Takata et al.* (1984) suggested two possibilities concerning the systematic relationships between *P. pungitius* and *P. sinensis* in the Omono River and those in Hokkaido. The first possibility is that such differences might be caused by a geo-clinal change in the degree of gene exchange between the two species from Hokkaido to the Tohoku District. The second possibility is that *P. pungitius* and/or *P. sinensis* in the Omono River belong to different species from those of Hokkaido, owing to a discontinuous change in the degree of gene exchange between the two species from Hokkaido to the Tohoku District. Takata (1986) showed that the populations of *P. pungitius* in the Omono River were genetically differentiated from that of Hokkaido or the Tohoku District. Therefore, the differences in genetic relationships between the Omono River and Hokkaido confirmed in the present study may be explained by the second possibility.

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雄物川水系におけるイバラトミヨ (*Pungitius pungitius*) とトミヨ (*P. sinensis*) の遺伝的差異

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雄物川水系平鹿の小さな池に同所的に生息しているイバラトミヨとトミヨの生殖的隔離の有無をアイソザイムを用いて調査した．鱗板形態から区分されたイバラトミヨとトミヨの集団間には，13遺伝子座中の1遺伝子座で対立遺伝子の置換が認められた．また，イバラトミヨに区分された集団（40個体）の中には，そのアイソザイム・パタンからトミヨとの雑種と推定される2個体が検出された．この2個体をイバラトミヨから除くと，両集団間には5遺伝子座で対立遺伝子の置換が存在することになる．従って，この池のイバラトミヨとトミヨには，かなり厳密な生殖的隔離が存在すると考えられた．また，雑種個体の出現頻度が低く，それらが F_1 雑種ではなくそれ以降の世代の雑種個体であったことから，両集団間には，交配前の隔離機構だけでなく，交配後の隔離機構として雑種崩壊も機能している可能性が示唆された．

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