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Biochemical Evidence for Reproductive Isolation between the Sympatric Populations of *Cottus amblystomopsis* and *C. nozawae*

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Abstract An electrophoretic study on the biochemical genetics of two sibling river-sculpins *Cottus amblystomopsis* and *C. nozawae* was undertaken with the primary objective of clarifying the reproductive isolation between the sympatric populations in three rivers around Cape Erimo of Hokkaido, where their distributions overlap widely along the river courses. At the 3 loci *Acp*, *Ldh* and *6Pgd*, out of 20 examined loci, evident displacement of alleles were observed between the two species. In addition, no genetical evidences for hybridization between the two species were detected in the three rivers examined. These results strongly suggest that the two species are reproductively isolated from each other even when they are distributed sympatrically and their distributions overlap widely along the course of a river.

Although it had been considered that the river sculpin *Cottus nozawae*, which is distributed in northern Honshu and Hokkaido of Japan, consists of a single biological species (Nakamura, 1963; Miyadi et al., 1976), Goto (1975a, b, 1977) found that there were two types which are morphologically very similar so that they can not be easily distinguished. The "small-egg type" mainly inhabits the lower course of rivers and the females spawn a large number of small-sized eggs, while the "large-egg type" is distributed mainly in the middle and upper courses of the river and the females spawn a small number of large-sized eggs. As for the life cycle, the small-egg type is amphidromous, while the large-egg type is fluvial (i.e., land-locked). After studies on geographic distribution and morphological variations, Goto (1980) proposed that the two types are different species and taxonomically the small-egg type should be identified with *Cottus amblystomopsis*, first reported by Schmidt (1904) from Sakhalin of the USSR, and that the species name *C. nozawae* should be aplied only to the large-egg type. Thereafter, some investigators have used these names to describe the two species of *Cottus* (Yabe, 1984; Hayashi et al., 1987).

In southern Hokkaido, *C. amblystomopsis* and *C. nozawae* are distributed separately in many coexisting rivers (lower reaches for the former species, and middle and upper reaches for the latter species), except the Hekiriji River where they have a narrow cohabiting area and appear to hybridize rarely (Goto, 1977). The two species are therefore almost completely isolated reproductively from each other (Goto et al., 1978; Goto, 1980, 1983). In addition, Goto (1983) demonstrated by mate preference tests that some incompatibility in the mating sequences exists between the two species, and suggested that the hybridization between them seldom occurs if ever they cohabit.

Recently, Kawamura (1982) reported that *C. nozawae* was distributed not only in the upstream area but also in the downstream area of steep and small rivers in the eastern Hidaka coast and Cape Erimo of Hokkaido. As a result, the longitudinal distribution of *C. nozawae* widely overlapped with that of *C. amblystomopsis*.

In the present paper we report the results of an electrophoretic survey designed to discriminate biochemically the individuals of *C. amblystomopsis* and *C. nozawae*, and the natural hybrids between them in three cohabiting rivers around Cape Erimo, Hokkaido. On the basis of the results, we estimate the extent of reproductive isolation between the sympatric populations of the two sibling species of *Cottus* and assess accurately the distribution patterns of the two species along the course of the rivers.

Materials and methods

Field collections of *C. amblystomopsis* and *C. nozawae* used in the present study were made in

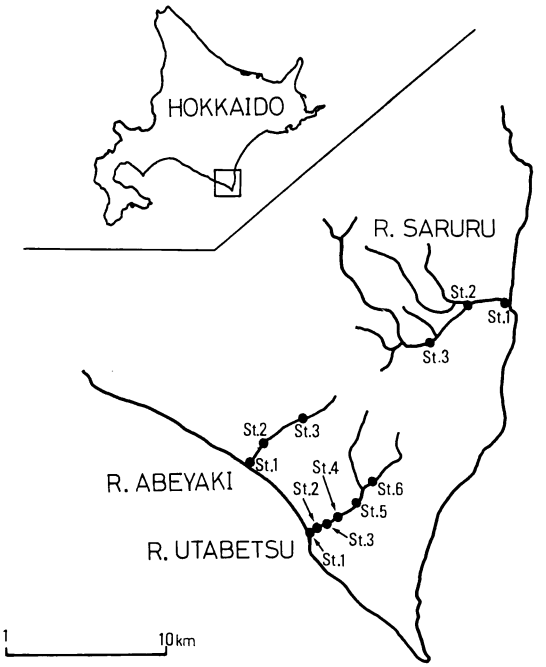

Fig. 1. Map showing examined rivers and sampling sites of the river-sculpins.

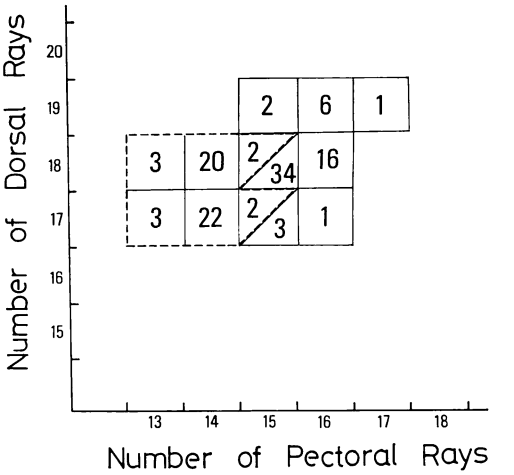

Fig. 2. Number of pectoral and dorsal rays in specimens of *Cottus amblystomopsis* (enclosed by solid line) and *C. nozawae* (enclosed by broken line) collected from St. 1 and St. 6 of the Utabetsu River, respectively. Figures indicate the number of individuals.

Table 1. Number of the specimens analysed.

	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	N
R. Abeyaki	36	32	34	—	—	—	102
R. Utabetsu	53	1	1	5	4	52	116
R. Saruru	34	34	26	—	—	—	94

Table 2. Enzyme systems and tissues analysed, electrophoretic buffer used, genetic loci coding to each enzyme system. C-AEA: citrate-aminopropyl diethanolamin buffer (Clayton and Tretiak, 1972); Ridgway: Ridgway buffer (Ridgway et al., 1970).

Enzyme	Isozyme	Tissue	Buffer-system	Locus
Acid phosphatase	ACP	liver	C-AEA	*Acp*
Glucosephosphate isomerase	GPI-I	liver, muscle	C-AEA	*Gpi*-I
	GPI-II	muscle	C-AEA	*Gpi*-II
	GPI-III	liver, muscle	C-AEA	*Gpi*-III
α-Glycerophosphate dehydrogenase	GPD-I	liver	C-AEA	*Gpd*-I
	GPD-II	muscle	C-AEA	*Gpd*-II
Isocitrate dehydrogenase	IDH-I	liver	C-AEA	*Idh*-I
	IDH-II	muscle	C-AEA	*Idh*-II
Lactate dehydrogenase	LDH	muscle	C-AEA	*Ldh*
Malate dehydrogenase	MDH-I	liver	C-AEA	*Mdh*-I
	MDH-II	liver	C-AEA	*Mdh*-II
	MDH-III	liver	C-AEA	*Mdh*-III
	MDH-IV	muscle	C-AEA	*Mdh*-IV
Malic enzyme	ME	muscle	C-AEA	*Me*
6-Phosphogluconate dehydrogenase	6PGD	liver	C-AEA	*6Pgd*
Sarcoplasmic protein	SP-I	muscle	Ridgway	*Sp*-I
	SP-II	muscle	Ridgway	*Sp*-II
	SP-III	muscle	Ridgway	*Sp*-III
Superoxide dismutase	SOD-I	liver	Ridgway	*Sod*-I
	SOD-II	muscle	Ridgway	*Sod*-II

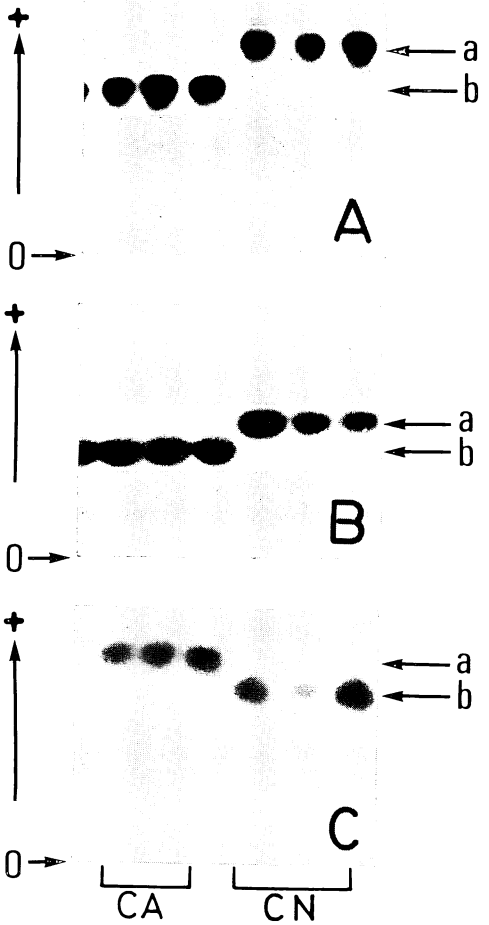

Fig. 3. Electrophoretic patterns of ACP in liver (A), LDH in muscle (B) and 6PGD in liver (C). CA, *Cottus amblystomopsis*; CN, *C. nozawae*.

three rivers, the Abeyaki, Utabetsu and Saruru rivers around Cape Erimo of Hokkaido, Japan (Fig. 1) in July and August 1986. In each of these rivers, 3 to 6 sampling sites were established along its course and sculpins were captured with dip and casting nets in each site (Fig. 1, Table 1). The collected samples were conveniently classified by their morphological characteristics according to the description by Goto (1975b, 1980). In the Utabetsu River, for example, sculpins collected from St. 1 and St. 6 were thought to correspond to *C. amblystomopsis* and *C. nozawae*, respectively, mainly due to their number of pectoral and dorsal fin rays (Fig. 2).

The specimens captured were immediately frozen with dry ice and stored in the laboratory at −20 to −80°C until electrophoretic analysis. Livers and skeletal muscles were removed from the frozen fish and minced with a small amount of distilled water. Cell-lysates or dip components were directly soaked by filter paper wick (Toyo Roshi Co. 51A) of suitable size. Horizontal starch gel electrophoresis was carried out at 4 mA/cm^2 for about 3 hours. Starch gel plates were prepared by adding 12% Amylan (Jookoo Industry Co.) to the appropriate gel buffer. The nine enzymes and one general protein examined were acid phosphatase (ACP), glucosephosphate isomerase (GPI), α-glycerophosphate dehydrogenase (GPD), isociterate dehydrogenase (IDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), 6-phosphogluconate dehydrogenase (6PGD), superoxide dismutase (SOD) and sarcoplasmic protein (SP). Two buffer systems described by Ridgway et al. (1970) for SOD and SP and by Clayton and Tretiak (1972) for the other 8 enzymes were used in the course of the present study (Table 2). The staining procedure followed Shaw and Prasad (1970) with slight modifications.

Results

Genetic control of isozymes and protein. Twenty gene loci coding for nine enzymes and one general protein were assumed in the present study (Table 2). The electrophoretic patterns and the assumption of loci and their alleles are briefly described below.

ACP: The ACP activity appeared in liver. *Acp* was represented by a single band migrating to the anode, but displacement of alleles was observed between the two species. *C. amblystomopsis* was fixed with *Acp*-a, migrating faster than *Acp*-b fixed in *C. nozawae* (Fig. 3A).

GPD: The products of the liver-specific locus *Gpd*-I were found to move rapidly toward the anode, and those of the muscle-specific locus *Gpd*-II migrated more slowly. *Gpd*-I was polymorphic with two alleles (a and b) in *C. amblystomopsis*, but was monomorphic and fixed with the b allele in *C. nozawae*. On the other hand, a common allele was observed at *Gpd*-II in both species.

IDH: The IDH activity was found also in two zones, one in the liver coded by *Idh*-I and the other in the muscle coded by *Idh*-II. In *Idh*-I, *C.*

amblystomopsis was polymorphic with two alleles (a and b), while *C. nozawae* was fixed with the b allele. The *Idh*-II was fixed with a common allele in both species.

LDH: The activity of this enzyme was detected in the muscle. *Ldh* was represented by a single band migrating to the anode, but displacement of alleles was observed between the two species. *C. nozawae* was fixed with *Ldh*-a, migrating faster than *Ldh*-b fixed in *C. amblystomopsis* (Fig. 3B).

MDH: The MDH activity appeared in four zones in the liver (loci *Mdh*-I, *Mdh*-II and *Mdh*-III) and muscle (locus *Mdh*-IV). Genetic divergence and variation were not observed on these loci.

ME: The activity of this enzyme was found in the muscle. *Me* was represented by a single band slightly migrating to the anode. Genetic differences were not observed on this locus.

6PGD: The 6PGD activity appeared in the

Table 3. Allele frequency at 20 loci in 6 populations of *Cottus amblystomopsis* (CA) and *C. nozawae* (CN). The figures in parentheses indicate the number of samples examined.

Locus	Allele	R. Abeyaki		R. Utabetsu		R. Saruru	
		CA (38)	CN (64)	CA (57)	CN (59)	CA (41)	CN (53)
Acp	a	0.000	1.000	0.000	1.000	0.000	1.000
	b	1.000	0.000	1.000	0.000	1.000	0.000
Gpi-I		1.000	1.000	1.000	1.000	1.000	1.000
Gpi-II		1.000	1.000	1.000	1.000	1.000	1.000
Gpi-III	a	0.000	1.000	0.000	1.000	0.000	0.295
	b	1.000	0.000	1.000	0.000	1.000	0.705
Gpd-I	a	0.013	0.000	0.018	0.000	0.000	0.000
	b	0.987	1.000	0.982	1.000	1.000	1.000
Gpd-II		1.000	1.000	1.000	1.000	1.000	1.000
Idh-I	a	0.276	0.000	0.330	0.000	0.359	0.000
	b	0.724	1.000	0.670	1.000	0.641	1.000
Idh-II		1.000	1.000	1.000	1.000	1.000	1.000
Ldh	a	0.000	1.000	0.000	1.000	0.000	1.000
	b	1.000	0.000	1.000	0.000	1.000	0.000
Mdh-I		1.000	1.000	1.000	1.000	1.000	1.000
Mdh-II		1.000	1.000	1.000	1.000	1.000	1.000
Mdh-III		1.000	1.000	1.000	1.000	1.000	1.000
Mdh-IV		1.000	1.000	1.000	1.000	1.000	1.000
Me		1.000	1.000	1.000	1.000	1.000	1.000
6Pgd	a	1.000	0.000	1.000	0.000	1.000	0.000
	b	0.000	1.000	0.000	1.000	0.000	1.000
Sp-I		1.000	1.000	1.000	1.000	1.000	1.000
Sp-II		1.000	1.000	1.000	1.000	1.000	1.000
Sp-III		1.000	1.000	1.000	1.000	1.000	1.000
Sod-I		1.000	1.000	1.000	1.000	1.000	1.000
Sod-II		1.000	1.000	1.000	1.000	1.000	1.000

Table 4. Genetic distance between the populations of same species and between *Cottus amblystomopsis* and *C. nozawae* in three rivers. CA, *C. amblystomopsis*; CN, *C. nozawae*.

		CA		CN		
		R. Utabetsu	R. Saruru	R. Abeyaki	R. Utabetsu	R. Saruru
CA	R. Abeyaki	0.0001	0.0004	0.2306	0.2306	0.1762
	R. Utabetsu	—	0.0001	0.2331	0.2331	0.1785
	R. Saruru	—	—	0.2342	0.2342	0.1796
CN	R. Abeyaki	—	—	—	0.0000	0.0254
	R. Utabetsu	—	—	—	—	0.0254

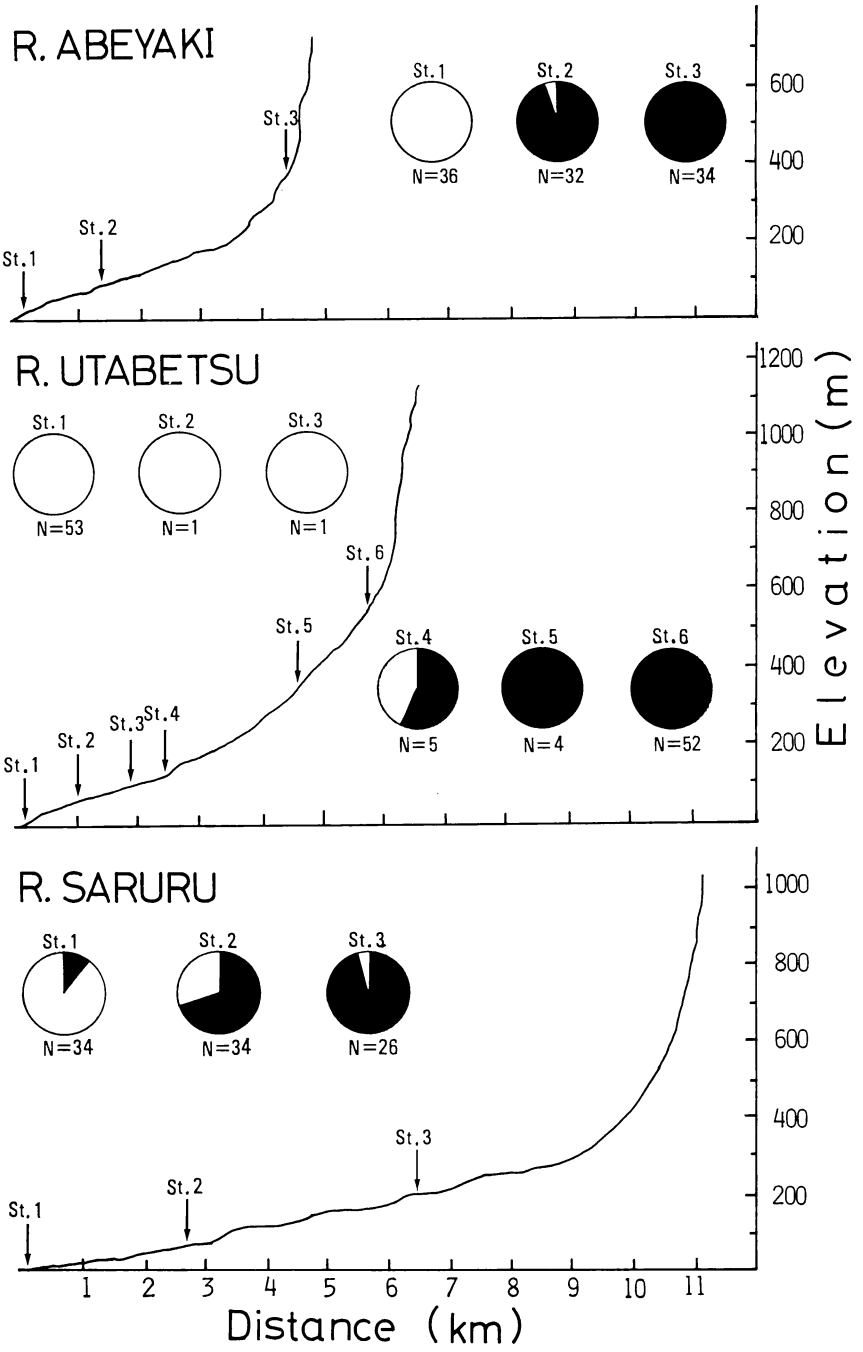

Fig. 4. Ratio of individuals of *Cottus amblystomopsis* (white circle) and *C. nozawae* (black circle) captured in each sampling site of the Abeyaki, Utabetsu and Saruru rivers.

liver, having a single band. In *6Pgd*, displacement of alleles was observed between the two species: *6Pgd*-a in *C. amblystomopsis*, migrating faster than *6Pgd*-b in *C. nozawae* (Fig. 3C).

GPI: The activity of this enzyme appeared both in the liver and muscle. The products of the loci *Gpi*-I and *Gpi*-III were found in both tissues, while those of *Gpi*-II were found only in

muscle. The single band coded by *Gpi*-II was observed at the same location to the anode with that of *Gpi*-I, and produced a hybrid band with the band migrating to the cathode and coded by *Gpi*-III. The *Gpi*-I and *Gpi*-II were fixed with a common allele in both species. In *Gpi*-III, *C. nozawae* was polymorphic with two alleles (a and b), while *C. amblystomopsis* was fixed with the b allele.

SOD: The SOD activity was found in two zones, one in the liver coded by *Sod*-I and the other in the muscle coded by *Sod*-II. The products of *Sod*-I moved faster to the anode than those of *Sod*-II. Genetic differences were not observed on these loci.

SP: Sarcoplasmic proteins were assumed to be controlled by three loci, *Sp*-I, *Sp*-II and *Sp*-III. The products of *Sp*-I had the highest mobility toward the anode, and those of the other two loci migrated more slowly. These three loci were fixed with a common allele in the two species.

Allele frequency. Observed frequencies of alleles in the six populations of the two species are summarized in Table 3. The phenotypic patterns for 14 out of 20 loci were monomorphic in the present populations: *Gpd*-II, *Idh*-II, *Mdh*-I, *Mdh*-II, *Mdh*-III, *Mdh*-IV, *Me*, *Gpi*-I, *Gpi*-II, *Sod*-I, *Sod*-II, *Sp*-I, *Sp*-II and *Sp*-III. The other 6 loci, *Acp*, *Gpd*-I, *Idh*-I, *Ldh*, *6Pgd* and *Gpi*-III, were polymorphic. Comparison of observed genotypic frequencies with Hardy-Weinberg expectations did not show statistically significant deviations in the locus *Idh*-I of either population of *C. amblystomopsis* nor in the locus *Gpi*-III of the Saruru River population of *C. nozawae*.

Displacement of alleles was observed at three loci (*Acp*, *Ldh* and *6Pgd*) between *C. amblystomopsis* and *C. nozawae* in all three rivers examined (Table 3). Significant differences in allelic frequency of the loci *Idh*-I and *Gpi*-III were also detected between the sympatric populations of the two species ($P<0.01$). Out of 102, 116 and 94 individuals collected from the Abeyaki, Utabetsu and Saruru rivers, respectively, no hybrid between the two species was observed in the present study.

Genetic differentiation. To estimate the degree of genetic divergence between *C. amblystomopsis* and *C. nozawae*, and between the populations of each species, genetic distance was calculated by the formula proposed by Nei (1972). Genetic distance ranged from 0.0002 to 0.0009 (average 0.0004) between populations of *C. amblystomopsis*, and from 0 to 0.0254 (average 0.0169) between populations of *C. nozawae* (Table 4). On the other hand, genetic distance between the two species ranged from 0.1757 to 0.2359 (average 0.2149).

Longitudinal distribution. On the basis of biochemical discrimination, the ratio of individuals of *C. amblystomopsis* and *C. nozawae* captured in each sampling site of the three rivers was accurately predicted (Fig. 4). In both the Abeyaki and Utabetsu rivers, the two species were distributed almost separately along the course of the river: *C. amblystomopsis* was distributed alone in the lower course (St. 1 for R. Abeyaki and St. 1–3 for R. Utabetsu), while *C. nozawae* was alone in the upper course (St. 3 for R. Abeyaki and St. 5–6 for R. Utabetsu), though they cohabited St. 2 of the Abeyaki River and St. 4 of the Utabetsu River. In the Saruru River, on the other hand, the two species coexisted in all three sites, though *C. amblystomopsis* was abundant in the lower course (St. 1) and *C. nozawae* was predominant in the upper course (St. 3).

Discussion

Through the present electrophoretic survey, it was strongly suggested that *C. amblystomopsis* and *C. nozawae* are reproductively isolated from each other in three rivers around Cape Erimo in Hokkaido, where Kawamura (1982) reported that the two species were distributed sympatrically and that their distribution overlapped widely along the river course. This is evident from the electrophoretic data, which demonstrated that the two species lack the ability for exchange of genes, even when they are distributed sympatrically, on account of evident displacement of alleles at three loci and also from the fact that there are no hybrids between them. It is reasonable to consider, therefore, that the small- and large-egg types of Goto (1975a) are not intraspecific variation but different species as previously pointed out by Goto (1975b, 1977, 1980). The values of the genetic distance between the two species ranged from 0.1757 to 0.2359 with an average of 0.2149, and was almost equivalent to that between congeneric species of several freshwater fishes as noted by Kijima et al. (1986). These ranged from ap-

proximately 0.214 for 2 species of *Moxostoma* (Buth, 1977) to an average of 0.627 for 10 species of *Lepomis* (Avice and Smith, 1974a, b). This may also support that they are distinct species. We referred to such systematic relationship in order to correct existing views that the small-egg and large-egg types are ecological types within a single species (Miyadi et al., 1976; Kawamura, 1982; Nagata and Miyamoto, 1986). Hereafter, the two types should be classified into two distinct species, *C. amblystomopsis* and *C. nozawae*, respectively, as proposed by Goto (1980).

According to Mayr (1970), reproductive isolating mechanisms are classified into two major mechanisms, those that prevent interspecific crosses (premating mechanisms) and those that reduce success of interspecific crosses (postmating mechanisms). Since the two mechanisms are thought to be able to develop independently in the process of speciation, Futuyma (1979) proposed that the reproductive isolation between closely related species occurs by either premating mechanisms only, postmating mechanisms only, or both premating and postmating mechanisms.

In the case of *C. amblystomopsis* and *C. nozawae*, it has been shown that the two sibling species are strictly reproductively isolated from each other in the coexisting rivers of southern Hokkaido where their distributions are almost or completely separate along the course (Goto, 1975a, 1980, 1983; Goto et al., 1978). Through the mate preference tests in conspecific and heterospecific combinations of the two species, Goto (1983) also demonstrated that some incompatibilities in the mating sequences exist between them. From these facts, he suggested that premating mechanisms, the geographical and ethological isolating ones, may act as effective mechanisms to prevent interbreeding between the two *Cottus* species, and that the most important isolating mechanism seems to be behavioral.

In the rivers around Cape Erimo examined in the present study, the two species were not isolated over a fairly wide area along the course (Fig. 4), thus, geographical and habitat isolations are not effective and should be eliminated from the important mechanisms. Therefore, ethological isolating mechanisms may serve as the most important devices for reproductive isolation between *C. amblystomopsis* and *C. nozawae* in these rivers. The facts that the artificial hybrids between the two species were able to grow with no abnormality and with satisfactory survival rates (Goto, unpublished data), suggesting that there seems to be no postmating mechanisms for reproductive isolation between them, may also support this view.

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Literature cited

Avice, J. C. and M. H. Smith. 1974a. Biochemical genetics of sunfish. I. Geographic variation and subspecific intergradation in the bluegill, *Lepomis macrochirus*. Evolution, 28: 42–56.

Avice, J. C. and M. H. Smith. 1974b. Biochemical genetics of sunfish. II. Genetic similarity between hybridizing species. Amer. Nat., 108: 458–472.

Buth, D. G. 1977. Biochemical identification of *Moxostoma rhothoecum* and *M. hamiltoni*. Biochem. Syst. Ecol., 5: 57–60.

Clayton, J. W. and D. N. Tretiak. 1972. Amine-citrate buffer for pH control in starch gel electrophoresis. J. Fish. Res. Bd. Can., 29(8): 1167–1172.

Futuyma, D. J. 1979. Evolutionary biology. Sinauer Assoc. Inc., Sunderland, x+565 pp.

Goto, A. 1975a. Ecological and morphological divergence of the freshwater sculpin, *Cottus nozawae* Snyder—I. Spawning behavior and process of the development in the post-hatching stage. Bull. Fac. Fish. Hokkaido Univ., 26(1): 31–37, pl.1. (In Japanese with English summary.)

Goto, A. 1975b. Ecological and morphological divergence of the freshwater sculpin, *Cottus nozawae* Snyder—II. Morphological comparison of adult fishes of small-egg and large-egg types and their distribution. Bull. Fac. Fish. Hokkaido Univ., 26(1): 39–48. (In Japanese with English summary.)

Goto, A. 1977. Some considerations on speciation and adaptation of the freshwater sculpins in Hokkaido. Japan. J. Michurin Biol., 13(1): 39–47. (In Japanese with English summary.)

Goto, A. 1980. Geographic distribution and variations of two types of *Cottus nozawae* in Hokkaido, and morphological characteristics of *C. amblystomopsis* from Sakhalin. Japan. J. Ichthyol., 27(2): 95–105.

Goto, A. 1983. Spawning habits and reproductive

isolating mechanism of two closely related river-sculpins, *Cottus amblystomopsis* and *C. nozawae*. Japan. J. Ichthyol., 30(2): 168–175.

Goto, A., T. Nakanishi, H. Utoh and K. Hamada. 1978. A preliminary study of the freshwater fish fauna of rivers in southern Hokkaido. Bull. Fac. Fish. Hokkaido Univ., 29(2): 118–130. (In Japanese with English summary.)

Hayashi, M., Y. Nagata, A. Goto and S. Nishijima. 1987. Field guide of freshwater fishes in Japan. Tokai Univ. Press, Tokyo, 186 pp. (In Japanese.)

Kawamura, H. 1982. Freshwater fishes in the eastern Hidaka coast and Cape Erimo in Hokkaido, Japan. Sci. Rep. Hokkaido Fish Hatchery, 37: 1–12. (In Japanese with English summary.)

Kijima, A., N. Taniguchi and A. Ochiai. 1986. Genetic divergence and morphological difference between the spotted and common mackerel. Japan. J. Ichthyol., 33(2): 151–161.

Mayr, E. 1970. Population, species, and evolution. Belknap of Harvard Univ. Press, Cambridge, 453 pp.

Miyadi, D., H. Kawanabe and N. Mizuno. 1976. Coloured illustrations of the freshwater fishes of Japan. Hoikusha, Osaka, 462 pp. (In Japanese.)

Nagata, M. and M. Miyamoto. 1986. The down-stream migration of chum salmon fry, *Oncorhynchus keta*, released into the Utabetsu River of eastern Hidaka in Hokkaido, and the estimation of predation amount of the fry by fresh water sculpin, *Cottus nozawae* Snyder. Sci. Rep. Hokkaido Fish Hatchery, 41: 13–22. (In Japanese with English summary.)

Nakamura, M. 1963. Keys to the freshwater fishes of Japan fully illustrated in colors. Hokuryukan, Tokyo, 260 pp. (In Japanese.)

Nei, M. 1972. Genetic distance between populations. Amer. Nat., 106: 283–292.

Ridgway, G. J., S. W. Sherburne and R. D. Lewis. 1970. Polymorphism in the esterases of Atlantic herring. Trans. Amer. Fish. Soc., 99(1): 147–151.

Schmidt, P. 1904. Fishes of eastern sea in Russian Empire. S. Petersburg, xi+466 pp. (In Russian.)

Shaw, C. R. and R. Prasad. 1970. Starch gel electrophoresis of enzymes—a complication of recipes. Biochem. Genet., 4(2): 297–320.

Yabe, M. 1984. The genus *Cottus*. Page 326, pl. 292 *in* H. Masuda, K. Amaoka, C. Araga, T. Uyeno and T. Yoshino, eds. The fishes of the Japanese Archipelago. English text and plates. Tokai Univ. Press, Tokyo.

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エゾハナカジカとハナカジカの同所的個体群間の生殖的隔離についての生化学的証拠

安藤　忠・後藤　晃

北海道日高地方の襟裳岬周辺の3河川において，流程分布が重複する姉妹種エゾハナカジカとハナカジカの間の生殖的隔離の程度を評価するために，アイソザイム遺伝子を指標にして集団遺伝学的調査を行った．その結果，調査した20遺伝子座のうち，*Acp*, *Ldh* および *6Pgd* の3遺伝子座において，両種間に対立遺伝子の置換が認められた．そして両種間の雑種個体は，調査した3河川のいずれにおいても，全く見いだされなかった．以上のことから，エゾハナカジカとハナカジカは，同所的に分布し流程分布が重複する場合にも，その間に遺伝子交流を全く欠き，強固な生殖的隔離が存在すると考えられる．このことは両者をそれぞれ独立種であるとする見解を支持する．

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