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Genetic Differentiation among Eight Color Types of the Freshwater Goby, *Rhinogobius brunneus*, from Western Japan

Yasuji Masuda, Takakazu Ozawa and Sumio Enami

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Abstract Genetic differentiation among eight color types of the freshwater goby, *Rhinogobius brunneus*, from the western part of Japan was investigated by using electrophoretic methods. Four sympatric types (Cross-band, Dark, Cobalt and Large-Dark (A) types) did not share alleles at between one and six loci out of 12 loci tested. No hybrid specimens were found among these types. The average genetic distances among these four types ranged from 0.13 to 0.72, which fall within the range of values among congeneric species of fishes. The average genetic distances among the other four types, Large-Dark (B), Orange, Shinji-Lake and Boso types, were only 0.01 to 0.03, and fall within the range of values among conspecific populations. These results suggest that the former four types are clearly discrete species and the latter four types may be considered as intraspecific variations of a fifth species.

The gobiid genus *Rhinogobius* is a common group of freshwater fishes in Japan. At present three species are recognized (Hayashi, 1984): *R. giurinus* (Rutter), *R. brunneus* (Temminck et Schlegel), and *R. flumineus* (Mizuno). Among these species, *R. brunneus* is the most abundant and well-known in the country, but its taxonomy is very confusing because of its wide color variations.

R. brunneus was first recorded by Gill in 1859 (Jordan and Snyder, 1901b) and since then, many species names have been assigned, e.g. *Rhinogobius nagoyae* by Jordan and Seal (1906), *Ctenogobius kurodai* and *Ctenogobius katonis* by Tanaka (1908), and *Rhinogobius fluviatilis* by Tanaka (1925). However, Jordan and Tanaka (1927) regarded these nominal species as a synonym of *Rhinogobius similis*, and Tomiyama (1936) as that of *Gobius similis*. Later, Takagi (1962) revised the name *Rhinogobius similis* into *R. brunneus*, which has since been generally adopted.

Mizuno (1960a, b) re-examined the gobies collected from the Japanese Archipelago and recognized two species, *R. similis* (=*R. brunneus*) and *Tukugobius flumineus* (=*R. flumineus*), on the basis of their morphological and ecological differences. Moreover, in 1967, *R. similis* was separated into two color types (Mizuoka, 1967), and since then 11 types have been identified (Nishijima, 1968; Mizuoka, 1974, 1978; Nakayama, 1975; Mizuno, 1976; Uehara, 1980; Iwata, 1981). However, the specific status of these color types remains uncertain.

Mizuoka (1976) compared the muscle and blood proteins of three species of *Rhinogobius* while Mizuno and Azakami (1981) studied courtship behavior among the color types of *R. brunneus* kept in an aquaria. Neither of these studies was able to resolve the taxonomic status of the color types.

This study examines the degree of genetic differentiation among the eight color types of *R. brunneus* and the other two species, *R. flumineus* and *R. giurinus*, and clarifies the taxonomic status of the color types by using electrophoretic techniques.

Materials and methods

Samples of the three species of the genus *Rhinogobius* were collected from 16 localities in the western part of Japan (Fig. 1). The samples collected were as follows: *R. brunneus*, five samples of Cross-band type (CR♯1–5), one of Dark type (DA♯1), three of Cobalt type (CO♯1–3), three of Large-Dark type A (LA♯1–3), three of Large-Dark type B (LB♯1–3), one of Orange type (OR♯1), one of Shinji-Lake type (SH♯1), and one of Boso type (BO♯1); *R. flumineus*, three samples (♯1–3); *R. giurinus*, five samples (♯1–5). Details of sampling locality, date of collection, number of fish examined and range in standard length are

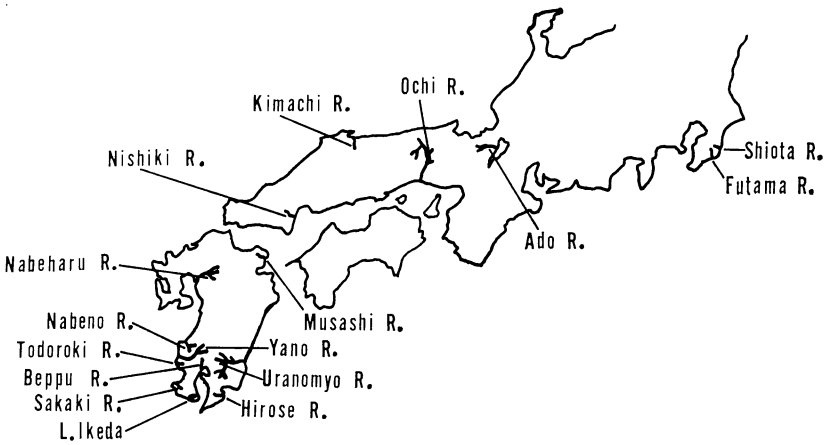

Fig. 1. Sampling localities of three species of *Rhinogobius*.

given in Table 1. All fishes were taken by scoop net and frozen immediately in dry ice before transporting to the Laboratory of Fisheries Resources, Kagoshima University. The specimens were stored at −30°C until tested.

Before electrophoresis, the specimens were thawed and identified as to species or type according to color pattern and external morpho-

Table 1. Sample data for three species of *Rhinogobius* used in electrophoretic analyses.

Species and type name	Locality	Date of collection	Number of fish	Standard length in mm
R. brunneus				
Cross-band Type #1	Hirose River, Kagoshima Pref.	Sep. 1980	26	39.7–64.8
#2	Yano River, Kagoshima Pref.	Jun. 1981	51	36.2–56.0
#3	Uranomyo River, Miyazaki Pref.	Aug. 1982	31	33.7–53.0
#4	Futama River, Chiba Pref.	Aug. 1982	28	33.7–48.4
#5	Beppu River, Kagoshima Pref.	Aug. 1983	44	39.9–58.0
Dark Type #1	Hirose River, Kagoshima Pref.	Sep. 1980	38	36.5–72.8
Cobalt Type #1	Hirose River, Kagoshima Pref.	Sep. 1980	36	40.6–81.3
#2	Nabeno River, Kagoshima Pref.	Sep. 1982	10	50.0–81.0
#3	Beppu River, Kagoshima Pref.	Aug. 1983	27	51.2–71.9
Large-Dark Type (A) #1	Hirose River, Kagoshima Pref.	Sep. 1980	20	38.2–61.3
#2	Uranomyo River, Miyazaki Pref.	Aug. 1982	41	31.6–64.4
#3	Beppu River, Kagoshima Pref.	Aug. 1983	51	40.0–68.5
Large-Dark Type (B) #1	Yano River, Kagoshima Pref.	Jun. 1981	52	39.0–63.6
#2	Nabeno River, Kagoshima Pref.	Sep. 1982	20	48.2–76.5
#3	Beppu River, Kagoshima Pref.	Aug. 1983	63	48.3–70.1
Orange Type #1	Ado River, Shiga Pref.	Aug. 1981	59	34.0–57.1
Shinji-Lake Type #1	Kimachi River, Shimane Pref.	Jul. 1982	24	44.1–66.3
Boso Type #1	Shiota River, Chiba Pref.	Aug. 1982	23	31.0–43.0
R. flumineus #1	Musashi River, Oita Pref.	Aug. 1980	27	28.7–48.5
#2	Nabeharu River, Fukuoka Pref.	May 1981	36	28.8–44.2
#3	Ochi River, Hyogo Pref.	Aug. 1981	37	30.5–42.6
R. giurinus #1	Nishiki River, Yamaguchi Pref.	Jul. 1980	44	39.2–53.1
#2	Hirose River, Kagoshima Pref.	Sep. 1980	91	36.5–66.1
#3	Todoroki River, Kagoshima Pref.	Sep. 1980	81	33.5–66.9
#4	Lake Ikeda, Kagoshima Pref.	Oct. 1980	95	37.9–58.5
#5	Sakaki River, Kagoshima Pref.	Jun. 1981	45	32.8–58.7

logical characters described by Mizuoka (1974, 1978), Mizuno (1976), Miyadi et al. (1976) and Hayashi (1984). For the electrophoretic analyses, the whole liver and small pieces of the lateral muscle were dissected and homogenized in an equal volume of distilled water. The homogenates were absorbed onto filter paper wicks (No. 51A, Toyo Filter Paper Co., Tokyo, Japan) and subjected to horizontal starch gel electrophoresis. Gels were prepared by using 12.5% Electrostarch (Electrostarch Co., Madison, Wisconsin, U.S.A.) and two buffer systems: CAEA (citric acid, N-(3-aminopropyl) diethanolamin), pH 7.0 and CAPM (citric acid, N-(3-aminopropyl) morpholine), pH 6.0 (Clayton and Tretiake, 1972).

The enzymes and proteins assayed, tissues examined, buffer systems and staining procedures used are presented in Table 2.

Loci and alleles were assigned each a number and a letter, respectively, in order of decreasing anodal mobility.

Results

Electrophoretic variation in enzymes and protein. Twelve gene loci coding for eight enzymes and general protein were scored in the three species of *Rhinogobius*. Relative mobility of allelic products and allele frequencies for each locus are given in Fig. 2 and Tables 3–5, respectively. The results for each enzyme and protein scored are described below:

Fumarate hydratase (FH): One *Fh* locus was scored in muscle extracts. Heterozygotes exhibited a clear five-banded pattern characteristic of tetramaric enzymes. In populations of *R. brunneus* and *R. flumineus* the Fh^a allele occurred at high frequency (>0.72), whereas *R. giurinus* was fixed for Fh^b allele.

Glucosephosphate isomerase (GPI): Two *Gpi* loci were observed in muscle extracts and the heterozygotes were three-banded for both loci. At the *Gpi*-1 locus, the populations of the CR, DA and CO types of *R. brunneus* carried the $Gpi\text{-}1^d$ allele in high frequency (>0.83), while the populations of the LA type were polymorphic for $Gpi\text{-}1^d$ and $Gpi\text{-}1^h$. In the LB, OR, SH and BO types of *R. brunneus*, $Gpi\text{-}1^h$ and $Gpi\text{-}1^e$ were common. Populations of *R. flumineus* were polymorphic for the three alleles ($Gpi\text{-}1^d$, $Gpi\text{-}1^h$ and $Gpi\text{-}1^i$) or fixed for $Gpi\text{-}1^d$. *R. giurinus* carried the $Gpi\text{-}1^g$ allele in high frequency (>0.97). At the *Gpi*-2 locus, the populations of CR and LA types of *R. brunneus* carried the $Gpi\text{-}2^g$ allele in high frequency (>0.96), whereas the populations of DA and CO types of *R. brunneus* had the $Gpi\text{-}2^d$ and $Gpi\text{-}2^e$ alleles in high frequency, respectively. In the LB, OR, SH and BO types of *R. brunneus*, the $Gpi\text{-}2^f$ allele was in high frequency (>0.70). The populations of *R. flumineus* were polymorphic for the three alleles ($Gpi\text{-}2^g$, $Gpi\text{-}2^a$ and $Gpi\text{-}2^e$) or fixed for the $Gpi\text{-}2^g$ allele. *R. giurinus* carried the $Gpi\text{-}2^i$ allele in high frequency (>0.92).

Isocitrate dehydrogenase (IDH): Two loci were observed for IDH. This enzyme is a dimer since

Table 2. Enzymes and proteins, tissue source, buffer systems and staining procedures used in electrophoretic analyses of three species of *Rhinogobius*.

Enzyme and protein (Abbreviation)	Symbol for locus	Tissue source	Buffer system	Reference for staining procedure
Fumarate hydratase (FH)	*Fh*	muscle	CAEA	Shaw and Prasad (1970)
Glucosephosphate isomerase (GPI)	*Gpi*-1	muscle	CAEA	Shaw and Prasad (1970)
	Gpi-2	muscle	CAEA	Shaw and Prasad (1970)
α-Glycerophosphate dehydrogenase (α-GPD)	*α-Gpd*	muscle	CAPM	Numachi (1971)
Isocitrate dehydrogenase (IDH)	*Idh*-1	liver	CAPM	Taniguchi and Numachi (1978)
	Idh-2	muscle	CAPM	Taniguchi and Numachi (1978)
Malate dehydrogenase (MDH)	*Mdh*	liver	CAPM	Numachi (1970)
Malic enzyme (ME)	*Me*	muscle	CAEA	Ayala et al. (1972)
Phosphoglucomutase (PGM)	*Pgm*	muscle	CAPM	Shaw and Prasad (1970)
Superoxide dismutase (SOD)	*Sod*	liver	CAEA	Numachi (1972)
General protein (PROT)	*Prot*-1	muscle	CAEA	Taniguchi and Konishi (1971)
	Prot-2	muscle	CAEA	Taniguchi and Konishi (1971)

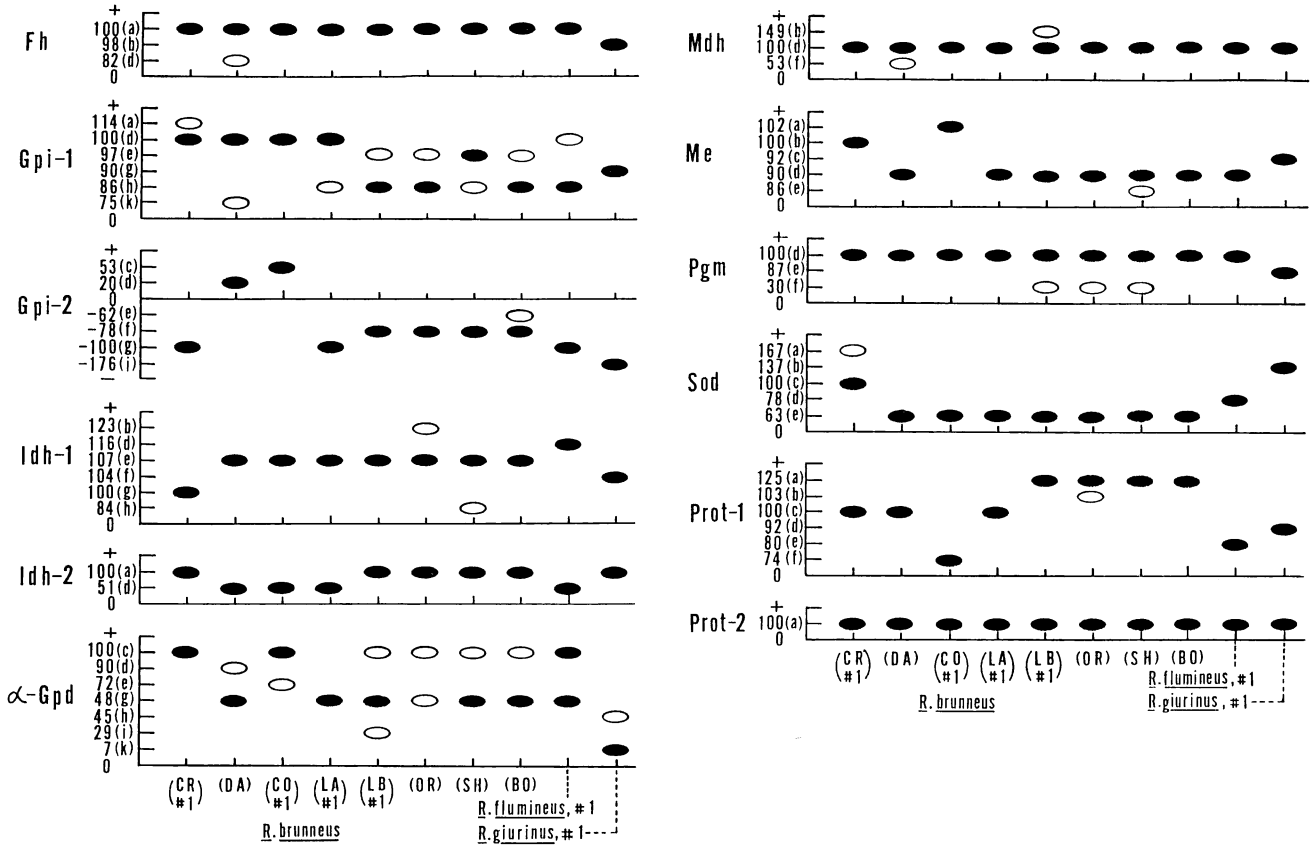

Fig. 2. Relative mobility of allelic products at 12 loci in selected populations of *Rhinogobius*. Mobility of the most common allele in Cross-band type (CR#1) is represented as 100. Allelic products at frequencies $\geqq 0.50$ are indicated by solid symbols. Those at frequencies <0.50 but $\geqq 0.05$ are indicated by hollow symbols, while those at frequencies <0.05 are excluded. Heterozygotes or heteropolymers are not included. CR, Cross-band type; DA, Dark type; CO, Cobalt type; LA, Large-Dark type (A); LB, Large-Dark type (B); OR, Orange type; SH, Shinji-Lake type; BO, Boso type.

heterozygotes exhibited a three-banded pattern. At the liver specific locus, *Idh*-1, populations of the CR type of *R. brunneus* were fixed for the *Idh*-1^{g} allele, whereas other types of *R. brunneus* carried the *Idh*-1^{e} allele in high frequency except for the OR type which was polymorphic for *Idh*-1^{e}, *Idh*-1^{b} and *Idh*-1^{a}. The populations of *R. flumineus* were fixed for *Idh*-1^{d} while those of *R. giurinus* carried the *Idh*-1^{f} allele in high frequency (>0.98). At the muscle-specific locus, *Idh*-2, all populations of *Rhinogobius* were essentially monomorphic for either *Idh*-2^{a} or *Idh*-2^{d}.

α-Glycerophosphate dehydrogenase (α-GPD): Only the α-Glycerophosphate dehydrogenase system with the least anodal mobility could be scored consistently in muscle extracts. Heterozygotes showed a three-banded pattern. Populations of the CR and CO types of *R. brunneus* carried the *α-Gpd*^{c} allele in high frequency, while the DA type was polymorphic for *α-Gpd*^{g} and *α-Gpd*^{d}. The populations of the LA type were fixed for *α-Gpd*^{g}, but the LB, OR, SH and BO

Table 3. Allele frequencies at *Fh*, *Gpi*-1, *Gpi*-2 and *Idh*-1 loci in three species of *Rhinogobius*. CR, Cross-band type; DA, Dark type; CO, Cobalt type; LA, Large-Dark type (A); LB, Large-Dark type (B); OR, Orange type; SH, Shinji-Lake type; BO, Boso type.

Population	*Fh*	*Gpi*-1	*Gpi*-2	*Idh*-1
R. brunneus				
CR#1	a	d (.94) a (.06)	g (.96) b (.02) j (.02)	g
CR#2	a	d (.96) h (.02) k (.01)	g	g
CR#3	a	d (.92) a (.08)	g	g
CR#4	a (.98) e (.02)	d (.89) b (.07) a (.02) h (.02)	g	g
CR#5	a	d (.97) h (.03)	g (.97) j (.03)	g
DA#1	a (.72) d (.28)	d (.83) k (.12) l (.04) i (.01)	d (.99) h (.01)	e
CO#1	a	d (.90) b (.04) h (.04) f (.01)	c	e
CO#2	a	d (.95) h (.05)	c	e
CO#3	a	d (.92) h (.06) f (.03)	c (.97) h (.03)	e
LA#1	a	d (.58) h (.42)	g	e (.98) b (.02)
LA#2	a (.94) e (.06)	d (.56) h (.44)	g	e (.99) b (.01)
LA#3	a	d (.39) h (.61)	g	e
LB#1	a	h (.58) e (.36) d (.04) c (.01) k (.01)	f	e
LB#2	a	h (.70) e (.25) b (.03) d (.03)	f	e
LB#3	a	h (.58) e (.31) d (.08) j (.03)	f	e
OR#1	a (.99) c (.01)	h (.66) e (.33) j (.01)	f (.99) i (.01)	e (.68) b (.31) a (.01)
SH#1	a	h (.44) e (.52) d (.04)	f	e (.94) h (.06)
BO#1	a	h (.72) e (.28)	f (.70) e (.30)	e
R. flumineus #1	a	d (.26) h (.70) l (.04)	g	d
#2	a	d	g	d
#3	a	d	g (.36) a (.62) e (.01)	d
R. giurinus #1	b	g	i (.99) f (.01)	f (.98) c (.02)
#2	b	g	i (.95) k (.05)	f
#3	b	g (.99) j (.01)	i (.93) k (.07) f (.01)	f (.99) c (.01)
#4	b	g	i	f (.99) h (.01)
#5	b	g (.97) j (.03)	i (.92) k (.08)	f

Table 4. Allele frequencies at *Idh*-2, *α-Gpd* and *Mdh* loci in three species of *Rhinogobius*.

Population	*Idh*-2	*α-Gpd*	*Mdh*
R. brunneus			
CR#1	a	c (.98) a (.02)	d
CR#2	a	c (.96) a (.03) b (.01)	d
CR#3	a	c (.95) a (.05)	d
CR#4	a	c (.88) a (.12)	d (.98) e (.02)
CR#5	a	c	d
DA#1	d (.97) b (.03)	g (.71) d (.29)	d (.91) f (.09)
CO#1	d	c (.82) e (.17) f (.01)	d
CO#2	d	c (.85) e (.15)	d
CO#3	d	c (.92) f (.06) e (.03)	d
LA#1	d	g	d
LA#2	d	g	d
LA#3	d	g	d (.94) f (.06)
LB#1	a	g (.68) c (.21) i (.05) d (.04) j (.01) l (.01)	d (.95) b (.05)
LB#2	a	g (.70) c (.15) d (.08) i (.08)	d (.98) b (.02)
LB#3	a	g (.42) c (.58)	d
OR#1	a	g (.49) c (.46) j (.03) e (.02)	d
SH#1	a (.98) c (.02)	g (.60) c (.35) e (.02) i (.02)	d
BO#1	a	g (.85) c (.15)	d
R. flumineus #1	d	g (.50) c (.50)	d
#2	d	g (.92) c (.08)	d
#3	d	g	d (.96) a (.03) f (.01)
R. giurinus #1	a	k (.95) h (.05)	d
#2	a	k (.94) h (.06)	d (.99) f (.01)
#3	a	k (.96) h (.04)	d (.98) f (.02) c (.01)
#4	a (.99) e (.01)	k	d
#5	a	k	d

Table 5. Allele frequencies at *Me*, *Pgm*, *Sod*, *Prot*-1 and *Prot*-2 loci in three species of *Rhinogobius*.

Population	*Me*	*Pgm*	*Sod*	*Prot*-1	*Prot*-2
R. brunneus					
CR#1	b	d (.98) b (.02)	a (.33) c (.67)	c	a
CR#2	b	d (.96) a (.04)	a (.70) c (.30)	c	a
CR#3	b	d (.95) b (.03) a (.02)	a (.23) c (.77)	c	a
CR#4	b	d (.91) b (.09)	a (.45) c (.55)	c	a
CR#5	b	d	a (.87) c (.13)	c	a
DA#1	d	d (.99) b (.01)	e	c (.99) a (.01)	a
CO#1	a	d	e	f	a
CO#2	a	d	e	f	a
CO#3	a (.72) c (.28)	d (.94) b (.03) f (.03)	e	f	a
LA#1	d	d	e	c	a
LA#2	d	d	e	c	a
LA#3	d	d	e	c	a
LB#1	d	d (.87) f (.11) a (.02)	e	a	a
LB#2	d	d (.80) f (.13) a (.08)	e	a	a
LB#3	d	d (.78) a (.22)	e	a	a
OR#1	d (.99) f (.01)	d (.91) f (.09)	e (.98) d (.02)	a (.93) b (.07)	a
SH#1	d (.81) e (.19)	d (.88) f (.12)	e	a (.96) b (.04)	a
BO#1	d	d	e	a	a
R. flumineus #1	d	d	d	e	a
#2	d	d	d	e	a
#3	d	d	d	e	a
R. giurinus #1	c	e	b	d	a
#2	c	e (.97) c (.02) b (.01)	b	d	a
#3	c	e (.96) c (.04)	b	d	a
#4	c	e	b	d	a
#5	c	e	b	d	a

types were polymorphic for α-*Gpd*^{g} and α-*Gpd*^{c}. Populations of *R. flumineus* were either polymorphic for α-*Gpd*^{g} and α-*Gpd*^{c} or fixed for α-*Gpd*^{g}. *R. giurinus* carried the α-*Gpd*^{k} allele in high frequency (>0.94).

Malate dehydrogenase (MDH): One *Mdh* locus was scored in liver extracts and heterozygotes could easily be distinguished by their three-banded pattern. All populations were essentially monomorphic for the *Mdh*^{d} allele.

Malic enzyme (ME): Two zones were observed in muscle extracts, but only the more anodal zone could be scored. Heterozygotes exhibited a clear five-banded pattern characteristic of tetrameric enzymes. Populations of the CR and CO types of *R. brunneus* carried unique alleles, *Me*^{b} and *Me*^{a}, respectively. The other types of *R. brunneus* and *R. flumineus* had a third allele, *Me*^{d}, while *R. giurinus* was fixed for another allele *Me*^{c}.

Phosphoglucomutase (PGM): One *Pgm* locus was scored in muscle extracts and heterozygotes were two-banded. All populations of *R. brunneus* and *R. flumineus* carried the *Pgm*^{d} allele in high frequency (>0.78), while *R. giurinus* was essentially monomorphic for *Pgm*^{c}.

Superoxide dismutase (SOD): One *Sod* locus was scored in liver extracts and heterozygotes were three-banded. Populations of the CR type of *R. brunneus* were polymorphic for *Sod*^{a} and *Sod*^{c}, whereas the other types of *R. brunneus* were fixed for *Sod*^{c} except for the OR type, which also carried the *Sod*^{d} allele in low frequency. *R. flumineus* and *R. giurinus* were fixed for *Sod*^{d} and *Sod*^{b}, respectively.

General protein (PROT): Two strongly stained zones were observed in muscle extracts and were scored as products of the two loci (*Prot*-1 and *Prot*-2). At the *Prot*-1 locus, heterozygotes exhibited a two-banded pattern. Populations of the CR and LA types of *R. brunneus* were fixed for *Prot*-1^{c} and the DA type carried this allele in high frequency (0.99). Populations of the CO type had a unique allele, *Prot*-1^{f}, while the LB, OR, SH and BO types carried *Prot*-1^{a} in high frequency (>0.93). *R. flumineus* and *R. giurinus* were fixed for *Prot*-1^{c} and *Prot*-1^{d}, respectively. At the *Prot*-2 locus, all populations were fixed for *Prot*-2^{a} allele.

Genetic distances among populations. To estimate the degree of genetic differentiation among populations, Nei's measurement of genetic distance was used (Nei, 1972). This was calculated directly from the allele frequencies shown in Tables 3–5. The average genetic distances among populations are presented in Table 6.

Among local populations of the same type or species, low average genetic distances were observed: 0.00–0.01 in each type of *R. brunneus* and *R. giurinus*, and 0.07 in *R. flumineus*.

Among the eight color types of *R. brunneus*, the average genetic distances ranged from 0.01 to 0.78. Low values (0.01–0.03) were observed between pairs of the LB, OR, SH and BO types, but high genetic distances (0.13–0.78) were recorded between the remaining pairs of the color types.

At the species level, average genetic distances among the three species were high (0.35–1.77); *R. giurinus* was more distant from the other species (Table 6, Fig. 3).

Discussion

Genetic differentiation among local populations. Since the technique of gel electrophoresis of protein was introduced to taxonomic studies, much information has been accumulated on the degree of genetic differentiation at various taxonomic levels (e.g. local populations, species and genera) of fishes. At the local population level, the average genetic distances ranged from 0.002 to 0.01 in marine fishes and from 0.002 to 0.13 in freshwater fishes on the basis of the following data: marine fishes, 0.002 in milkfish *Chanos chanos* (Winans, 1980), 0.008 in anemonefish *Amphiprion clarkii* (Bell et al., 1982), 0.01 in flatfish (Ward and Galleguillos, 1983), 0.0028 in masu salmon *Oncorhynchus masou* (Okazaki, 1986) and 0.0020 in red seabream *Pagrus major* (Taniguchi et al., 1986); freshwater fishes, 0.027 in bluegill *Lepomis macrochirus macrochirus* and 0.004 in *L. m. purpurescens* (Avise and Smith, 1977), 0.13 in stoneroller *Campostoma anomalum* and 0.03 in *C. oligolepis* (Buth and Burr, 1978), 0.0017 in ayu *Plecoglossus altivelis* (Taniguchi et al., 1983) and 0.056 in Japanese dace *Tribolodon hakonensis* (Hanzawa et al., 1988). Thus, in general, the average genetic distances among local populations of freshwater fishes tend to be higher than in marine fishes, since the degree of geographical isolation in freshwater fishes is usually higher than in marine fishes.

Table 6. Average genetic distances among three species of *Rhinogobius*. Calculations are based on allele frequency data in Tables 3–5. Ranges are shown in parentheses.

Species and type name	*R. brunneus*								*R. flumineus*	*R. giurinus*
	CR	DA	CO	LA	LB	OR	SH	BO		
R. brunneus										
CR Type	0.01									
	(0.00–0.04)									
DA Type	0.72	—								
	(0.71–0.72)	—								
CO Type	0.70	0.41	0.01							
	(0.68–0.73)	(0.40–0.41)	(0.00–0.01)							
LA Type	0.58	0.13	0.43	0.00						
	(0.56–0.60)	(0.12–0.14)	(0.41–0.45)	(0.00–0.00)						
LB Type	0.76	0.43	0.59	0.36	0.01					
	(0.71–0.81)	(0.42–0.45)	(0.53–0.63)	(0.34–0.39)	(0.00–0.02)					
OR Type	0.70	0.46	0.59	0.39	0.02	—				
	(0.70–0.71)	—	(0.58–0.60)	(0.38–0.39)	(0.02–0.02)	—				
SH Type	0.73	0.44	0.56	0.38	0.01	0.02	—			
	(0.72–0.73)	—	(0.55–0.57)	(0.37–0.38)	(0.01–0.02)	—	—			
BO Type	0.78	0.40	0.60	0.31	0.02	0.03	0.03	—		
	(0.77–0.78)	—	(0.60–0.60)	(0.31–0.32)	(0.01–0.03)	—	—	—		
R. flumineus	0.71	0.49	0.67	0.35	0.70	0.67	0.70	0.64	0.07	
	(0.66–0.78)	(0.44–0.56)	(0.66–0.69)	(0.32–0.40)	(0.64–0.75)	(0.60–0.71)	(0.66–0.73)	(0.60–0.68)	(0.04–0.11)	
R. giurinus	1.35	1.77	1.72	1.77	1.33	1.29	1.31	1.33	1.77	0.00
	(1.33–1.37)	(1.77–1.78)	(1.62–1.78)	(1.76–1.80)	(1.32–1.34)	(1.29–1.30)	(1.30–1.31)	(1.33–1.34)	(1.74–1.79)	(0.00–0.00)

In this study, the average genetic distances between pairs of local populations within *R. brunneus* and within *R. giurinus* were very low (0.00–0.01), while that within *R. flumineus* was higher (0.07) (Table 6). As compared with the average genetic distances described above, the former values are typical of those in marine fishes and the latter in freshwater fishes. These results may reflect ecological differences among the three species: *R. brunneus* and *R. giurinus* usually migrate between river and sea according to their development (amphidromous type), while *R. flumineus* is typically a river fish (fluvial type) (Miyadi et al., 1976; Hayashi, 1984). Thus, the degree of gene mixing among local populations of *R. brunneus* and of *R. giurinus* is expected to be higher than that of *R. flumineus*.

Genetic differentiation among color types. The average genetic distances between pairs of the LB, OR, SH and BO types were low (0.01–0.03), while those between the remaining pairs of the color types were high (0.13–0.78) (Table 6). Shaklee et al. (1982) reviewed the genetic distances in many marine and freshwater fishes and found out that the average genetic distance between conspecific populations was 0.05 (range 0.002–0.065) and that between species was 0.30 (0.025–0.609). Similarly, Thorpe (1983) examined the distributions of Nei's genetic identity (I) for a large number of conspecific populations and congeneric species of various organisms and found out that the I values between most pairs (98%) of conspecific populations were above 0.9, whereas between congeneric species most (99.5%) were below 0.9. Transforming these I values to genetic distances (D) by using the formula, $D=-\log_e I$, D was found to be less than 0.105 between conspecific populations and more than 0.105 between congeneric species. As compared with these genetic distances, the present values between pairs of the LB, OR, SH and BO types fall within the range of conspecific populations and the values between the remaining pairs of the color types fall within the range of congeneric species.

Table 7. Number of loci at which the four sympatric types of *Rhinogobius brunneus* (Hirose River) did not share alleles. Gene loci examined=12.

Type name	CR	DA	CO	LA
CR	—	6	6	5
DA		—	4	1
CO			—	4
LA				—

Table 7 shows the number of loci at which the four sympatric types (CR, DA, CO and LA types caught in Hirose River) did not share alleles. On the basis of this table, these four types did not share alleles at between one and six loci out of the 12 examined. Moreover, no hybrid specimens have been detected in these sympatric populations. These findings indicate that the four types of *R. brunneus* maintain separate gene pools and are reproductively isolated.

Mizuno et al. (1979) investigated the ecological differences of these four sympatric types (CR, DA, CO and LA types) and observed clear habitat segregation among them. Moreover, the four types are quite distinct in coloration, while the other four types (LB, OR, SH and BO types) closely resemble each other (Mizuoka, 1974; Mizuno, 1976; Uehara, 1980). Mizuno and Azakami (1981) examined the courting behavior among the six color types of *R. brunneus* in an aquaria. They observed frequent courting between OR and SH types, and infrequent courting between CR, DA, CO, LA and OR (or SH) types.

From these results, the CR, DA, CO and LA types of *R. brunneus* are clearly discrete species and the other four types, LB, OR, SH and BO, may be considered as intraspecific variations of a fifth species.

Among these discrete species, the CR, DA and LA color types have been described as different species (Mizuno, 1976): the CR type as *Rhinogobius nagoyae* by Jordan and Seale (1906), the DA type as *Ctenogobius similis* by Jordan and Snyder (1901a), and the LA type as *Rhinogobius fluviatilis* by Tanaka (1925). Scientific names of the other color types await further morphological studies and search of original descriptions.

In the Ryukyu Archipelago, three more color types of *R. brunneus* have been reported (Nishijima, 1968; Nakayama, 1975; Iwata, 1981; Hayashi, 1984). To establish the taxonomy of these types, further electrophoretic analyses are needed.

Genetic differentiation among species. The average genetic distances among the three species of the genus *Rhinogobius* were high (0.35–1.77), especially for *R. giurinus* which showed considerably higher values (>1.29) than the other two species (Table 6). In Fig. 3, the phenetic rela-

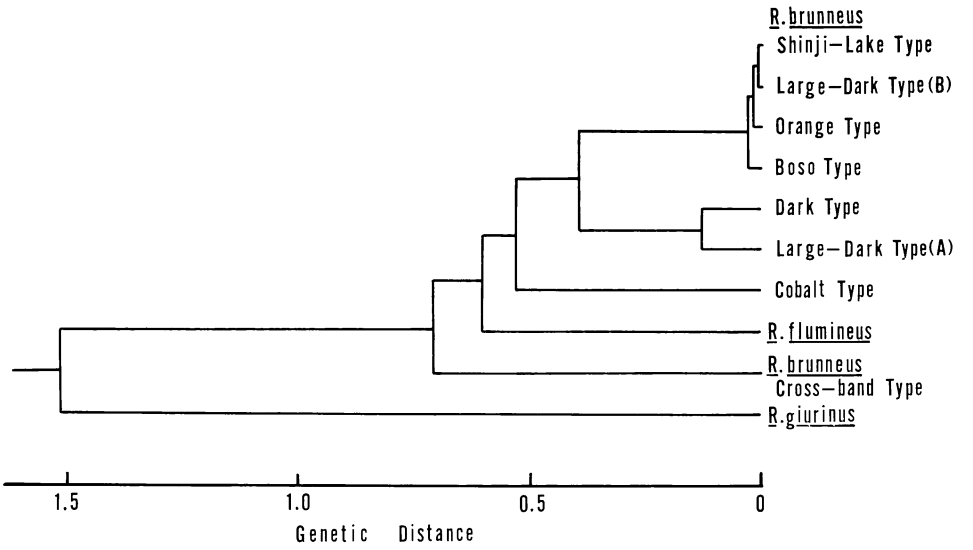

Fig. 3. Dendrogram of *Rhinogobius* populations based on UPGMA clustering (Sneath and Sokal, 1973) of the genetic distance data in Table 6.

tionships of the three species are illustrated: the close relationship between *R. brunneus* and *R. flumineus*, and their apparent separation from *R. giurinus*. These relationships are consistent with the observations of haemoglobin banding patterns of the three *Rhinogobius* species by Mizuoka (1976).

On the basis of morphological and ecological studies of *Rhinogobius* species, *R. giurinus* differs from *R. brunneus* and *R. flumineus* in the following characters (Miyadi et al., 1976): scales on the occipital region are present in *R. giurinus* but absent in *R. brunneus* and *R. flumineus*; a pair of red-bands on the snout of *R. brunneus* and *R. flumineus* is absent in *R. giurinus*; and *R. giurinus* inhabits estuaries or lower reaches of rivers while *R. brunneus* and *R. flumineus* inhabit middle to upper reaches of rivers.

Thus, the genetical, morphological, and ecological data indicate that *R. flumineus* and the eight color types of *R. brunneus* constitute one group and are clearly different from *R. giurinus*.

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(Faculty of Fisheries, Kagoshima University, Kagoshima 890, Japan)

ヨシノボリ8型の遺伝的分化

増田育司・小沢貴和・江波澄雄

西日本の河川域から採集したヨシノボリ8型について電気泳動法による遺伝的比較を行った．その結果，同一河川で採集した横斑型・黒色型・るり型・黒色大型Aの4型は，分析した12遺伝子座のうち1-6遺伝子座で異なった遺伝子を保有し，かつ各型間の交雑個体は1個体も見出されなかった．これら4型間の遺伝距離は0.13-0.72であり，魚類の同属種間で得られる値の範囲内にあった．一方，黒色大型B・橙色型・宍道湖型・房総型の4型間の遺伝距離は0.01-0.03と低く，同種個体群間で得られる値の範囲内にあった．これらの結果から，前4型は明らかに別種であり，後4型は同一種（第5番目の種）に属するものと思われる．

(890 鹿児島市下荒田 4-50-20 鹿児島大学水産学部)