Journal of National Fisheries University 45 (2) 87−93 (1996)

Genetic variability and differentiation of a freshwater goby *Odontobutis obscura* from Yamaguchi Prefecture*[1]

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Genetic variability and differentiation were analyzed on 12 populations of the Donko *Odontobutis obscura* from Yamaguchi Prefecture. Genetic variations were electrophoretically determined for 18 allozyme-coding loci, in terms of proportion of polymorphic loci (PL), average heterozygosity (H_o and H_e), F_{ST} and G_{ST} values, and Nei's genetic distance (D). The study group exhibited higher variability (PL=0.241, H_o=0.083, H_e=0.092) and differentiated populations (F_{ST}=0.062～1.000, G_{ST}=0.551) as compared to other fish groups, even among intertributary populations (G_{ST}=0.120～0.137). The Tama River population, the eastern-most population from the Japan Sea slope of Yamaguchi Prefecture, was distantly related to the other populations (D=0.463).

1 Introduction

The Donko *Odontobutis obscura* (Temminck et Schlegel) (Odontobutidae : Gobioidei) is a common freshwater goby occurs in Yamaguchi and other western prefectures of Japan[1]. It deposits larger eggs than most other freshwater gobies which exhibit an amphidromous life history mode[2]. The large egg size yields large larvae and results in a resident fluvial life strategy[3]. The migration within a river course was thought to be restricted[2], therefore, the Donko is an excellent object to study fish population differentiation and freshwater biogeography. The present study examines the genetic variability, geographic differentiation, and population structure of the Donko from Yamaguchi Prefecture based on specific allozyme genetic indices.

2 Materials and methods

Twelve samples were collected from 1992 to 1994 (Table 1 and Fig. 1). Populations 3-5 (TABE, SKYK and KRKW) and 7-9 (TATN, KRMK and ABU) were from three different tributaries of the Koya River system and the Abu River system, respectively. The fish were frozen and stored at -70℃ until processed for horizontal starch-gel electrophoresis (12% gel)[4,5]. The 12 enzymes, 18 loci, tissues, and buffer systems are listed in Table 2. Locus and gene nomenclature follows

水産大学校研究業績 第1553号、1996年5月29日受付
Contribution from National Fisheries University, No.1553. Received May 29, 1996
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Table 1. Sample data of 12 populations of *Odontobutis obscura* from Yamaguchi Prefecture

Population No.	River (River system)	Abbreviation	Date of collection	Sample size
1.	R. Kawatana	KWTN	Jun. 1993	25
2.	R. Ayaragi	AYRG	Jul. 1993	15
3.	R. Tabe (R. Koya)	TABE	Apr. 1994	25
4.	R. Sakogayoku (R. Koya)	SKYK	Sep. 1992	18
5.	R. Kurokawa (R. Koya)	KRKW	Jun. 1993	9
6.	R. Yae (R.Kotou)	KTOU	Apr. 1992	15
7.	R. Tateno (R. Abu)	TATN	Sep. 1993	6
8.	R. Kurameka (R. Abu)	KRMK	Dec. 1993	12
9.	R. Abu	ABU	Dec. 1993	3
10.	R. Ooi	OOI	Dec. 1993	5
11.	R. Tama	TAMA	Sep. 1993	9
12.	R. Nishiki	NSHK	Sep. 1993	8

Table 2. Emzymes, enzyme numbers (E.C.), loci, tissues and buffer systems used

Enzyme (E.C.)	Locus	Tissue	Buffer
Aspartate arinotransferase (2.6.1.1)	*AAT-1**	Liver	TC
Alcohol dehydrogenase (1.1.1.1)	*ADH**	L	AC
Creatine Kinase (2.7.3.2)	*CK**	Muscle	RW
Glycero-3-phosphate dehydrogenase (1.1.1.8)	*G3PDH**	M	TC
Glucose-6-phosphate isomerase (5.3.1.9)	*GPI-1**	M	RW
	*GPI-2**	M	RW
Isocitrate dehydrogenase (1.1.1.42)	*IDHP-1**	L, Eye	TC
	*IDHP-2**	M, E	TC
L-Lactate dehydrogenase (1.1.1.27)	*LDH-1**	E	TC
	*LDH-2**	M, E	TC
	*LDH-3**	E	TC
Malate dehydrogenase	*MDH-1**	M	TC
	*MDH-2**	M, L	TC
	*MDH-3**	M	TC
Phosphogluconate dehydrogenase (1.1.1.44)	*PGDH**	E, L	TC
Phosphoglucomutase (5.4.2.2)	*PGM-2**	M	RW
Superoxide dismutase (1.15.1.1)	*SOD**	L	RW
Xanthine dehydrogenase (1.1.1.204)	*XDH**	L	RW

TC : Tris-citrate buffer[6] (pH 8.0, diluted 1 : 9 for the gel), 4mA/cm² for 4h. AC : Amine (N-(3-aminopropyl)-morpholine) citrate buffer[7] (pH 6.0), 4mA/cm² for 3h. RW : Discontinuous Tris-citric acid (gel pH 8.5), lithium hydrooxide-boric acid (tray pH 8.5) buffer[8], 4mA/cm² for 2h.

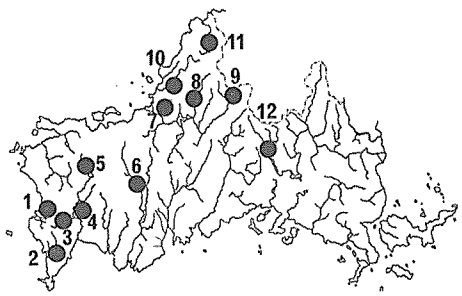

Fig. 1 Map of Yamaguchi prefecture showing sample localities of *Odontobutis obscura*. Locality numbers correspond to sample numbers in Table 1.

the system of Shaklee et al[9]. Different loci of the same enzyme system are given Arabic numerals from anodic to cathodic ones. The most common allele at a locus scored in the Kotou River population (6 KTOU) was designated as * *100*[10] and the other alleles were labeled according to the relative mobility. The calculated genetic indices include proportion of polymorphic loci (PL, most common allele does not exceed 0.95), observed and expected heterozygosity (H_o and H_e), fixation index (F_{ST}), coefficient of gene differentiation (G_{ST}), and Nei's[11] unbiased genetic distance (D). Genetic variability data (PL, H_o and H_e) were obtained from six different populations with more than 10 individuals. Genetic differentiation data (F_{ST} and G_{ST}) were calculated from all 12 populations and from each three tributary populations of the Koya and Abu Rivers. Allele frequency homogeneity was examined utilizing log-likelihood ratios (G-test) among the three tributary populations in both rivers. A dendrogram of D was drawn by the UPGMA method[12].

3 Results

Alleles on *AAT-1**, *CK**, *LDH-1**, *LDH-3**, *MDH-3**, and *XDH** were uniform through 12 populations surveyed. Allelic frequencies of the remaining 12 loci are presented in Table 3. Eleven populations, excluding the Tama River population (11 TAMA), exhibited the most and/or the second most frequent alleles in common. Alleles on six loci, *G3PDH**, *GPI-1**, *IDHP-1**, *IDHP-2**, *MDH-1**, and *SOD**, were notably displaced and represent a diagnostic difference between the TAMA population and the other 11 populations. Mean values of PL, Ho, He, and H_o/H_e for 6 populations were 0.241, 0.083, 0.092, and 0.891, respectively (Table 4). The H_o/H_e ratios indicated homozygote excess in five out of these six populations, though no populations displayed significant departure from Hardy-Weinberg equilibria (chi-square test at $p > 0.05$). F_{ST} value ranged 0.062-1.000 in the total populations, 0.000-0.474 in the Koya River populations, and 0.000-0.266 in the Abu River populations (Table 5). G_{ST} value scored 0.551 in the total populations, 0.120 in the Koya River populations, and 0.137 in the Abu River populations (Table 5).

The allele frequency differences among three tributary populations from the Koya and Abu Rivers were significant (G-test, $p < 0.001$ in both cases).

Genetic distance data (D) between pairs of 12 populations are described in Table 6. The dendrogram of D values is illustrated in Fig. 2. Eleven populations, other than the TAMA population, formed a cluster at $D = 0.075 \pm 0.038$ SD. In the cluster, each of three tributary populations from the Koya and Abu Rivers did not connect as neighbors. The TAMA population related with the other 11 populations at $D = 0.463 \pm 0.051$ SD.

4 Discussion

The Donko, in this study, expressed much greater genetic variability (Table 4) than other resident fluvial fish, such as the Kawayoshinobori *Rhinogobius flumineus* ($PL = 0.102$, $H_e = 0.037$)[13], a freshwater goby, the Hanakajika *Cottus nozawae* ($PL = 0.027$, $H_e = 0.009$)[14], a freshwater sculpin, and the freshwater form of the three spine sticklebacks Gasterosteus aculeatus ($PL = 0.039$, $H_o = 0.008$)[15]. The heterozygosity of the Donko was higher than that of American cyprinid fish (mean $H_e = 0.052$, 69 species)[16]. Species of freshwater fish are reported to exhibit less genetic variability than marine and diadromous species [14,15,17,18], probably due to the smaller effective size of population[19]. However,

Table 3. Alleles (allelic frequencies in parentheses) at 12 polymorphic loci in 12 populations of *Odontobutis obscura* from Yamaguchi Prefecture.

	1 KWTN	2 AYRG	3 TABE	4 SKYK	5 KRKW	6 KTOU	7 TATN	8 KRMK	9 ABU	10 OOI	11 TAMA	12 NSHK
*ADH**	**-100*(.680) **-106*(.320)	**-100*	**-100*	**-100*	**-100*	**-100*	**-100*(.667) **-146*(.333)	**-100*(.667) **-146*(.333)	**-100*	**-100*(.600) **-146*(.400)	**-100*	**-100*
*G3PDH**	**100*	**100*(.500) **76*(.500)	**100*(.980) **76*(.020)	**100*	**100*(.889) **76*(.111)	**125*(.033) **100*(.967)	**100*	**100*	**100*	**100*	**76*	**100*
*GPI-1**	**100*	**100*	**100*	**100*	**100*	**100*	**100*(.917) **92*(.083)	**100*	**100*	**100*	**105*	**100*
*GPI-2**	**115*(.400) **100*(.600)	**115*(.933) **100*(.067)	**115*(.960) **100*(.040)	**115*(.361) **100*(.639)	**115*	**100*(.900) **75*(.100)	**100*	**115*(.583) **100*(.417)	**115*(.167) **100*(.833)	**115*(.400) **100*(.600)	**100*	**115*(.062) **100*(.938)
*IDHP-1**	**100*	**100*	**100*	**100*	**100*	**100*	**100*	**100*	**100*	**100*	**109*	**100*
*IDHP-2**	**132*(.100) **100*(.900)	**100*	**132*(.120) **100*(.880)	**100*	**100*	**132*(.467) **100*(.533)	**132*(.417) **100*(.583)	**100*	**100*	**100*	**114*	**132*(.167) **100*(.833)
*LDH-2**	**100*	**100*	**100*	**100*	**100*	**100*(.900) **45*(.100)	**100*	**100*	**100*	**100*	**100*	**100*
*MDH-1**	**100*	**100*	**100*	**100*	**100*	**100*	**100*	**100*	**116*(.333) **100*(.667)	**100*	**80*	**100*
*MDH-2**	**130*(.360) **100*(.640)	**130*(.633) **100*(.367)	**130*(.280) **100*(.720)	**130*(.333) **100*(.667)	**130*(.333) **100*(.667)	**130*(.333) **100*(.667)	**130*(.417) **100*(.583)	**130*(.417) **100*(.583)	**130*(.667) **100*(.333)	**130*(.400) **100*(.600)	**130*	**130*(.187) **100*(.813)
*PGDH**	**100*	**100*	**100*(.840) **83*(.160)	**100*	**100*	**100*	**100*	**100*	**100*	**100*	**100*	**100*
*PGM-2**	**100*(.180) **90*(.820)	**107*(.033) **100*(.967)	**100*	**100*	**100*	**100*(.900) **90*(.100)	**100*	**100*	**100*	**100*	**100*	**100*
*SOD**	**100*(.500) **42*(.500)	**42*	**100*(.500) **42*(.500)	**100*(.722) **42*(.278)	**100*(.556) **42*(.444)	**100*(.833) **42*(.167)	**100*	**100*	**100*	**100*	**124*	**100*

Table 4. Proportion of polymorphic loci and average heterozygosity in 6 populations of *Odontobutis obscura* analized more than 10 individuals

Population No. Abbr.	Polymorphic loci*1	Average heterozygosity Observed(Ho)	 Expected(He)*2	Ho/He	Sample Size
1. KWTN	.389	.142	.148	.959	25
2. AYRG	.222	.067	.084	.798	15
3. TABE	.222	.076	.083	.916	25
4. SKYK	.167	.068	.073	.932	18
6. KTOU	.278	.086	.084	1.024	15
8. KRMK	.167	.056	.078	.718	12
Mean±SD	.241±.084	.083±.031	.092±.028	.891±.112	

*1 : A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
*2 : Biased estimate (Nei, 1978).

Table 5. F_{ST} and G_{ST} values for total 12 populations, 3 subpopulations from the Koya River, and 3 subpopulations from the Abu River

Loci	Total	Koya subpopulations	Abu subpopulations
F_{ST}			
*ADH**	.298	-	-
*G3PDH**	.711	.009	-
*GPI-1**	.617	-	.000
*GPI-2**	.519	.474	.262
*IDH-1**	1.000	-	-
*IDH-2**	.497	.065	.266
*LDH-2**	.062	-	-
*MDH-1**	.786	-	.125
*MDH-2**	.162	.000	.000
*PGDH**	.140	.095	-
*PGM-2**	.799	-	-
*SOD**	.610	.003	-
G_{ST}	.551	.120	.137

Table 6. Nei's unbiased genetic distance between pairs of 12 populations of *Odontobutis obscura* from Yamaguchi Prefecture

Population	2	3	4	5	6	7	8	9	10	11	12 NSHK
1. KWTN	.107	.068	.052	.073	.084	.081	.067	.077	.065	.544	.073
2. AYRG		.037	.075	.034	.159	.137	.089	.120	.109	.432	.137
3. TABE			.026	.000	.069	.078	.025	.065	.042	.526	.064
4. SKYK				.024	.016	.020	.006	.012	.009	.456	.011
5. KRKW					.071	.081	.020	.060	.038	.510	.066
6. KTOU						.000	.024	.001	.022	.378	.011
7. TATN							.027	.013	.022	.420	.002
8. KRMK								.012	.005	.479	.018
9. ABU									.012	.408	.014
10. OOI										.483	.015
11. TAMA											.459

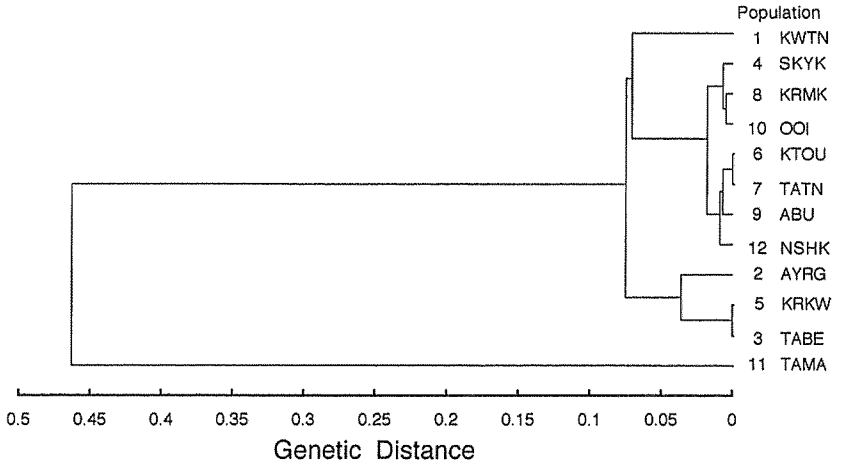

Fig. 2 Dendrogram (UPGMA) of Nei's unbiased genetic distance among 12 populations of *Odontobutis obscura* from Yamaguchi prefecure. Locality numbers and population abbreviations correnpond to in Table 1.

the Donko also indicated higher heterozygosity than marine fish species (mean H_o =0.055, 106 species)[20].

The reason why homozygotes exceeded in some populations (Table 4) is not obvious. As the heterozygote excess condition is also observed in populations from other areas (Sakai, unpublished data), the homo as well as heterozygote excess conditions may be stochastic in Donko.

The F_{ST} and G_{ST} values reflect the level of differentiation among populations. The more differentiated freshwater fish displays higher value (mean G_{ST} =0.325, 12 species) than the less differentiated diadromous fish (mean G_{ST} =0.066, 7 species) and marine fish (mean G_{ST} =0.045, 16 species)[17,18]. The Donko, in this study, as well as the Kawayoshinobori[13], expressed a higher differentiation level than other freshwater fish. Even among tributary populations, the Donko was differentiated considerably (Table 2). The allele frequency homogeneity test indicated that the tributary populations were heterogeneous. These results suggests that both the tributary population size and the introgression among tributary populations are restricted. The restricted migration within a river course has been discussed previously[2].

The genetic distance between the TAMA population and the other populations (Table 6, Fig. 2) extended to the intra-specific level (D=0.025-0.609, mean 0.30), well beyond the inter-specific values calculated for other fishes (D=0.002-0.065)[21]. This indicates the existence of biogeographically different groups of the Donko in Yamaguchi Prefecture. The TAMA population is related to the eastern populations along the Japan Sea slope (Sakai, unpublished data). The genetic differentiation data above suggest that the effective population size of the Donko is small. Contrarily, the genetic variability data may support the alternative state. The relatively high variability within a population may be due to the introgressive hybridization among subpopulations[22]. However, the introgression among subpopulations was determined to be restricted. This paradox may be solved, considering the Donko river populations are segregated into small tributary subpopulations. The introgression among subpopulations is usually restricted resulting in genetic differentiation. Subpopulation isolation is disrupted by intense typhoon caused river flooding. The resultant introgression between differentiated subpopulations results in increased subpopulation variability.

Acknowledgments

We are indebted to Dr. N. Takeshita, and Mrs. K. Kamiyama, H. Sugiura, T. Takahashi, T. Morimoto, K. Yoshikawa, N. Hoshino, Y. Yoshiwara, T. Noda, K. Matsuno, Y. Dogawa, and A. Michiyama, for their help in collecting the materials. Special thanks are go to Dr. Ira Levine, Coastal Plantation International, Inc., for reviewing the manuscript.

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山口県産ドンコの遺伝的変異性と分化

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山口県産ドンコ *Odontobutis obscura* 12集団における遺伝的変異性と分化を，電気泳動により検出されるアロザイム18遺伝子座に基づき，多型率（PL），平均ヘテロ接合体率（Ho, He），固定指数ならびに遺伝子分化指数（F_{st}, G_{st}），および遺伝的距離（D）によって検討した。山口県産ドンコは，他の魚類に比較して高い変異性（PL＝0.244，H_o＝0.075，H_e＝0.094）と分化程度（F_{st}＝0.062～1.000，G_{st}＝0.551）を示し，同一河川内の支流集団間でもよく分化していた（G_{st}＝0.120～0.137）。日本海側東端の田万川産業団は，他の集団から遺伝的に大きく離れていた（D＝0.463）。