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Genetic Variation of the Chinese Mudskipper, *Periophthalmus cantonensis* (Osbeck, 1762) (Pisces; Perciformes, Gobiidae) from Taiwan[1]

Jung-Ti Chang and Sin-Che Lee[2]

Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan 115, R.O.C.

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Jung-Ti Chang and Sin-Che Lee (1994) Genetic variation of the Chinese mudskipper, *Periophthalmus cantonensis* (Osbeck, 1762) (Pisces; Perciformes, Gobiidae) from Taiwan. *Zoological Studies* **33**(1): 34-43. The degree of genetic divergence of two hundred and seventy eight specimens of the Chinese mudskipper, *Periophthalmus cantonensis* (Osbeck, 1762) collected from six locations in Taiwan was estimated using starch gel electrophoresis. Fourteen enzymes corresponding to 22 gene loci and 47 alleles were recognized. An average Nei's genetic identity of $\bar{I} = 0.996$ (0.993-0.999) indicated highly homogenous protein structure. The proportion of polymorphic loci (0.95 level) varied from 0.0909 to 0.2727 with a mean of 0.1742; expected heterozygosity ranged from 0.028 to 0.065 with a mean of 0.051. The mean F_{st} differentiation among populations was only 0.0235. However, observed population phenotype numbers partly disagreed with Hardy-Weinberg equilibrium; this is probably due to some factors that affect the disequilibrium.

Key words: Chinese mudskipper population, Isozyme polymorphism, Genetic variation and environmental variability correlation.

The Chinese mudskipper, *Periophthalmus cantonensis* (Osbeck, 1762) is taxonomically placed in the family Gobiidae under Perciformes. The number of rays in each fin are: first dorsal XIV, second dorsal-I, 12, anal-I, 11, pectoral 14, ventral fin I, 5. The first dorsal fin upper margin is laterally rounded. The fish is covered with scattered black dots and has 26 vertebrae. This species is closely related to *P. vulgaris* of Japan. The concave upper margin on the first dorsal fin and one ray less on the second dorsal and anal fins distinguish it from *P. cantonensis*. No sensory pattern interspecific differences were found.

P. cantonensis is prolific in mangrove and mudflat estuaries in southern China and southeastern Asia (Gordon et al. 1985) and is sometimes found in southern Japan. In Taiwan this species is found at nearly all sandy coast estuaries (an exception is the extreme southern coasts); a small population is found in the Penghu Islands.

P. cantonensis is an euryhaline amphibious species, predominantly preying on insects and aquatic crustaceans. It is capable of skin respiration while exposed to air; its eurythermal tolerance is 30° to 35°C, euryhaline tolerance, 15 to 30‰.

Since *P. cantonensis* is primarily a estuarine microhabitat resident which does little immigration or emmigration, from individual habitats that are generally isolated. Gel electrophoresis (Ayala et al. 1974) permits allelic variation identification at a single gene locus. The gene locus can be selected for study without prior knowledge of its variance within or between populations due to wide distribution, eurythermal tolerance, and euryhaline tolerance. The objects of the present study are (1) the level of degree of interpopulational genetic variation estimation and (2) population heterozygosity to possible environmental parameter mean degree correlation.

1. This is contribution no. 384 of the Institute of Zoology, Academia Sinica, ROC.
2. To whom reprint requests should be sent.

MATERIALS AND METHODS

During May 1989 and April 1991, a total of 278 mudskipper specimens were collected from six locations (range of body length and mean): 31 from Tanshui (2.86-5.55 cm, $\bar{X}$ 4.20 SL), 50 from Ilan (3.25-6.80 cm, $\bar{X}$ 4.77 SL), 70 from Tainan (2.77-6.36 cm, $\bar{X}$ 5.00 SL), 46 from Tachia (3.05-5.57, $\bar{X}$ 4.38 cm), 36 from Tadu (3.06-5.77, $\bar{X}$ 4.58 cm), and 45 from Penghu (3.30-7.43, $\bar{X}$ 6.38 cm).

The samples were transferred to the laboratory and deep-frozen at −75°C until the experiment. The heart, eye, skeletal muscle, and liver were removed from each individual and homogenized separately in 2 volumes of chilled tris buffer (0.1 mM Tris-HCL, pH 7.0,with 1 mM EDTA and 0.05 mM NADP). The homogenates were centrifuged at 13,500 rpm for 40 minutes at 4°C. The supernatants were again stored at −75°C until electrophoresis. Horizontal electrophoresis of tissue extracts with 12% starch gel was performed. Three buffer systems were used during this series of experiments, continuous Tris-citrate (Siciliano and Show 1976) run at 280 V and 70 mA for 6 hr; continuous Tris-versene-borate (Siciliano and Show 1976) run at 180 V and 50 mA for 18 hr; and the discontinuous Tris-citric-boric-LiOH (Redfield and Salini 1980) run at 180 V and 50 mA for 18 hr.

The enzymes examined and buffers employed are tabulated in Table 1. The staining procedures followed those of Siciliano and Show (1976), Pasteur et al. (1988), and Murphy et al. (1990). Genetic identities and distances were computed according to Nei (1972) and Rohlf (1988). Protein-coding loci genetic nomenclature followed those of Shaklee et al. (1989). The most common allele is designated as 100, with other allele designations based on mobility relative to the most common allele. Electrophoretic data were tested for goodness of fit according to the Hardy-Weinberg hypothesis with X^2 values. The proportion of polymorphic loci (polymorphic loci/total loci, 0.95 level) and heterozygosity of individual populations were also compared (Ferguson 1980). Degree of genetic differentiation between populations was measured by Wright's F_{st} (the FIXATION INDEX, Wright 1978), which is equal to $s^2/p(1-p)$, where s^2 is the observed variance of allele frequencies among populations and p is the average of allele frequency over all populations.

RESULTS

Twenty-two loci and 47 allelic products were detected, among them the nineteen loci listed in

Table 1. List of enzyme systems, tissues, and buffers

Enzyme	E.C. number	Locus	Tissue	Buffer[1]
Alcohol dehydrogenase	1.1.1.1	*ADH**	Liver	LiOH
Aspartate aminotransferase	2.6.1.1	*sAAT**	Muscle	TVB
		*mAAT**		
Creatine kinase	2.7.3.2	*CK-A**	Liver	LiOH
		*CK-B**		
α-Glycereophosphate dehydrogenase	1.1.1.8	*α-GPDH**	Muscle	TVB
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6PDH**	Muscle	TVB
Glucose phosphate isomerase	5.3.1.9	*GPI-A**	Muscle	LiOH
		*GPI-B**	Liver	
Lactate dehydrogenase	1.1.1.27	*LDH-A**	Muscle	TC
		*LDH-B**	Heart	
		*LDH-C**	Eye	
Malate dehydrogenase	1.1.1.37	*MDH-A**	Muscle	TC
		*MDH-B**		
Malic enzyme	1.1.1.40	*ME**	Muscle	TVB
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	Muscle	TVB
Phosphoglucomutase	2.7.5.1	*PGM-A**	Muscle	TVB
		*PGM-B**		
6-Phosphogluconate dehydrogenase	1.1.1.44	*6-PGDH**	Eye	TC
			Muscle	
Sorbital dehydrogenase	1.1.1.14	*SDH**	Liver	LiOH
Superoxide dismutase	1.15.1.1	*SOD-1**	Liver	LiOH
		*SOD-2**		

[1]The tris-versene-borate buffer (TVB) pH 8.0 and the tris-citrate (TC) pH 7.0; Siciliano and Show (1976).
The tris-citric-boric-LiOH buffer (gel, pH 8.31; electrode, pH 8.26); Redfield and Salini (1980).

Table 2. Allele frequencies and genetic differentiation at 19 loci in six populations of *Periophthalmus cantonensis*[1]

Locus	Allele	TN(70)	PH(45)	IL(50)	TS(31)	TC(46)	TU(36)	F_{st}
*sAAT**	*129*	0.014	0	0.010	0.032	0.054	0.014	0.0154
	100	0.986	1.000	0.990	0.968	0.946	0.986	0.0154
*mAAT**	*315*	0	0	0	0.016	0	0	0.0135
	100	1.000	1.000	1.000	0.984	1.000	1.000	0.0132
*ADH**	*100*	0.993	1.000	0.989	1.000	1.000	1.000	0.0064
	60	0.007	0	0.011	0	0	0	0.0064
*CK-A**	*125*	0	0	0	0	0	0.043	0.0360
	112	0.027	0	0.031	0	0	0.057	0.0241
	100	0.973	1.000	0.969	1.000	1.000	0.900	0.0489
*CK-B**	*100*	1.000	1.000	0.980	1.000	1.000	1.000	0.0169
	95	0	0	0.020	0	0	0	0.0169
*G6PDH**	*130*	0.029	0	0.042	0.032	0.155	0.061	0.0477
	100	0.971	1.000	0.958	0.968	0.845	0.939	0.0477
*GPI-A**	*118*	0.044	0.190	0.032	0.017	0.033	0.125	0.0579
	100	0.875	0.780	0.904	0.931	0.837	0.778	0.0267
	80	0.081	0.030	0.064	0.052	0.130	0.097	0.0148
*GPI-B**	*200*	0.007	0	0.022	0	0	0.014	0.0098
	100	0.993	1.000	0.978	1.000	1.000	0.986	0.0098
*LDH-A**	*100*	0.993	1.000	1.000	1.000	0.989	1.000	0.0065
	65	0.007	0	0	0	0.011	0	0.0065
*LDH-B**	*100*	0.985	1.000	0.950	0.984	1.000	1.000	0.0235
	60	0.015	0	0.050	0.016	0	0	0.0235
*LDH-C**	*100*	0.991	1.000	0.990	0.983	0.978	1.000	0.0068
	96	0.009	0	0.010	0.017	0.022	0	0.0068
*MDH-A**	*100*	1.000	1.000	0.990	1.000	1.000	1.000	0.0081
	80	0	0	0.010	0	0	0	0.0081
*MDH-B**	*125*	0.007	0	0	0.017	0	0	0.0101
	100	0.993	1.000	1.000	0.983	1.000	1.000	0.0101
*MPI**	*104*	0.007	0	0.030	0.017	0	0	0.0141
	100	0.971	1.000	0.910	0.966	0.978	0.986	0.0263
	86	0.022	0.000	0.060	0.017	0.022	0.014	0.0153
*PGM-A**	*116*	0.007	0	0	0.019	0	0	0.0116
	100	0.993	1.000	1.000	0.962	1.000	1.000	0.0260
	94	0	0	0	0.019	0	0	0.0160
*PGM-B**	*100*	0.978	1.000	1.000	0.981	1.000	0.986	0.0098
	75	0.022	0	0	0.019	0	0.014	0.0098
*6-PGDH**	*113*	0.071	0.078	0.030	0.016	0.043	0.042	0.0106
	100	0.914	0.922	0.950	0.984	0.957	0.903	0.0134
	89	0.014	0	0.020	0	0	0.055	0.0264
*SDH**	*113*	0	0.045	0	0	0	0	0.0379
	100	0.729	0.943	0.670	0.921	0.652	0.614	0.0912*
	70	0.271	0.012	0.330	0.079	0.348	0.386	0.1103*
*SOD-2**	*136*	0.016	0	0.070	0	0.065	0	0.0379
	100	0.984	1.000	0.930	1.000	0.935	1.000	0.0379
Observed heterozygosity		0.0470	0.0202	0.0527	0.0322	0.0477	0.0486	
Expected heterozygosity		0.0530	0.0276	0.0642	0.0336	0.0636	0.0652	
Proportion of polymorphic loci		0.1364	0.1364	0.2727	0.0909	0.2273	0.1818	

[1]TN, Tainan; PH, Penghu; IL, Ilan; TS, Tanshui; TC, Tachia; TU, Tadu. Numbers in the parentheses indicate sample size. Asterisks other than those for loci indicate higher genetic differentiation.

Table 2 were polymorphic, the remaining three were monomorphic loci; *α-GPDH**, *ME**, and *SOD-1**, and are excluded from the table. All the loci mentioned above posess high mobility and clear resolution after tissue (muscle, heart, eye, and liver) specific examination. Figure 1 shows locus assignment and allelic variations indicating polymorphisms among individuals. The AAT has two loci, the cytosolic *sAAT** and mitochondrial *mAAT**, are both actively expressed in muscle tissue, with

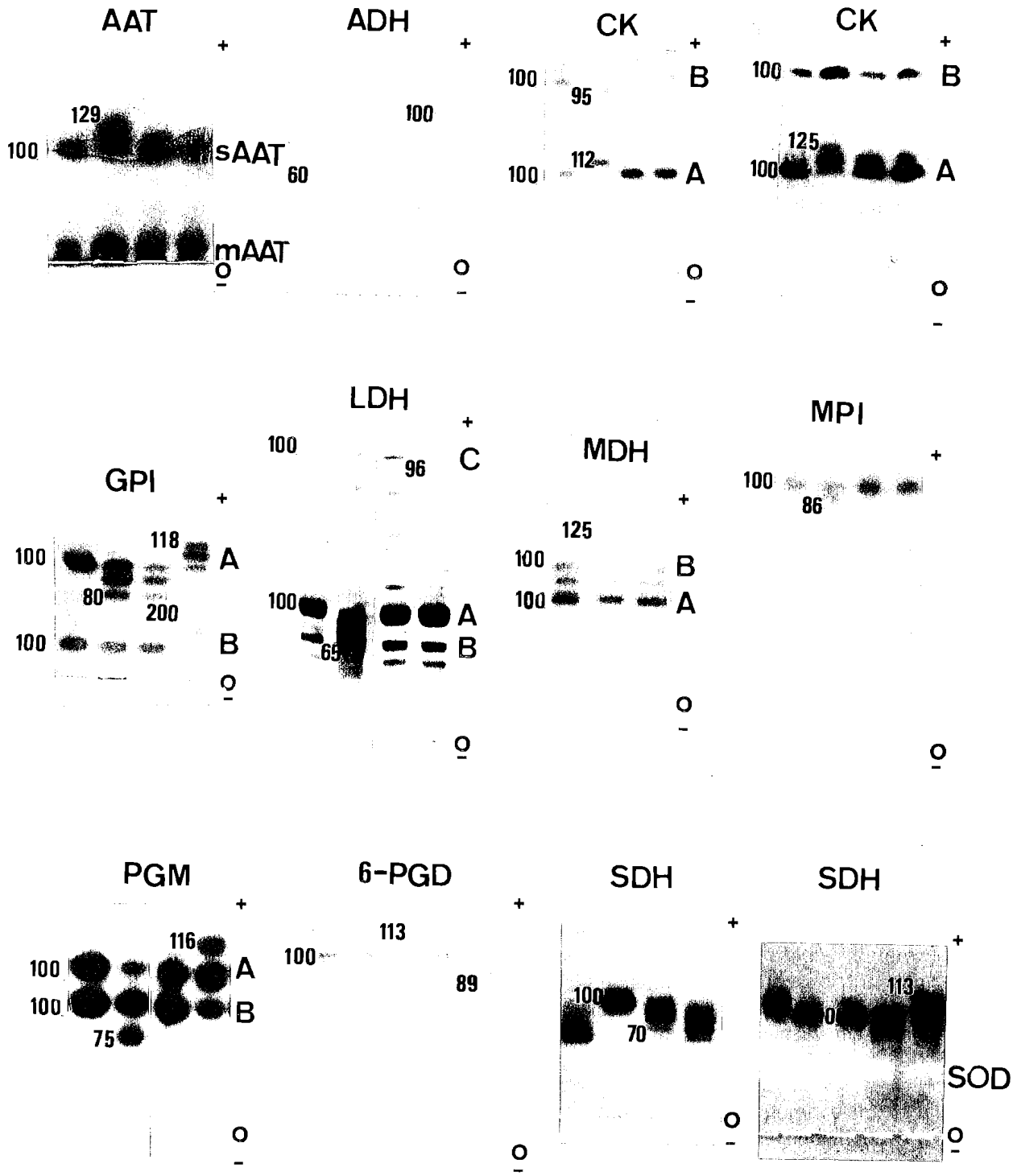

Fig. 1. Isozyme patterns of heterogenous loci from six populations.
AAT, Ilan population muscle tissue; ADH, Tainan population liver tissue; CK, Ilan and Tadu population liver tissue; GPI, Tadu population liver tissue; LDH, Tachia population eye tissue; MDH, Tanshui population muscle tissue; MPI, Tachia population muscle tissue; PGM, Tainan population muscle tissue; 6-PGDH, Ilan population eye tissue; SDH, Tachia and Penghu population liver tissue and SOD appears as light bands and is resolved on SDH enzyme. o, sample origin; +, anode; −, cathode.

two alleles at each locus. The ADH has only one locus, with two alleles in liver tissue, having three-band heterozygotes in some individuals. The CK has two loci, *CK-A** and *CK-B**, expressed actively in muscle and eye tissues, respectively. Both loci are equally expressed in the liver where some heterozygotes have three bands in *CK-A**. The GPI has two loci, the *GPI-A** locus with three alleles while the *GPI-B** locus with two alleles are expressed actively in liver tissue. All six populations are polymorphic at these 2 loci. Among the three alleles at locus *GPI-A**: allele *100* is most dominant in all six populations, allele *118* frequency is higher in the Penghu and Tadu populations (19%) and (12.5%), respectively; but lower in Tanshui (1.7%), Ilan (3.2%), Tachia (3.3%), and Tainan (4.4%). Additionally allele *80* is more common in Tachia (13%), Tadu (9.7%), Tainan (8.1%), Ilan (6.4%), Tanshui (5.2%), and less common in the Penghu population (3%) (Fig. 2). G6PDH has one locus, *G6PDH** with two alleles is active in muscle tissue. The *LDH-A**, *LDH-B** and *LDH-C** loci all appeared in eye tissue, though *LDH-A** with 2 alleles is the strongest in muscle tissue, *LDH-B** also with two alleles in the heart. The above three loci exhibit five banded heterozygotes. Additional intermediate bands are produced by the interaction of gene product among *LDH-A**, *B** and *C** loci. MDH has two loci, *MDH-A** and *MDH-B**, both are expressed in muscle tissue. The *MDH-B** locus exhibited three-banded heterozygotes. The MPI has one locus with three alleles, which is active in muscle tissue. The PGM has two loci, *PGM-A** and *PGM-B** in muscle tissue, with heterozygotes at both loci. The *PGM-A** locus has three alleles and the *PGM-B** locus has two alleles. The 6-PGDH has one locus with three alleles (*113, 100, 89*) which is active in eye tissue, three-banded heterozygotes were found in some individuals. Allele *100* is one of the most dominant alleles in all six populations and allele *89* is absent from the Penghu, Tachia, and Tanshui populations. The proportion of allele *113* is higher in the Penghu (7.8%), Tainan (7.1%), Tachia (4.3%), and Tadu (4.2%) populations and is lower in the Tanshui (1.6%), and Ilan (3%) populations (Fig. 3). SDH has one locus with three alleles which is actively expressed in liver tissue showing five-banded heterozygotes in all populations. The SOD has two loci, *SOD-1** and *SOD-2**, both are strongly active in liver tissue. They appeared simultaneously when stained for ADH, SDH and α-GPDH. The *SOD-1** locus displayed mono-

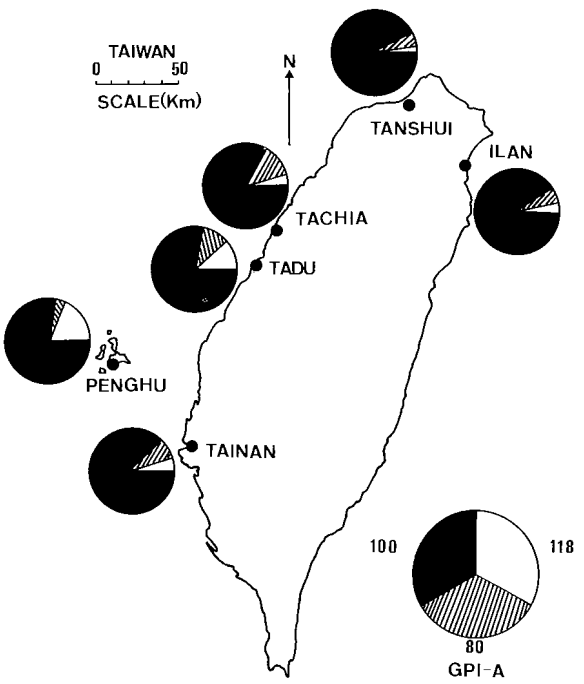

Fig. 2. Allelic frequencies at the *GPI-A** locus in six populations of *Periophthalmus cantonensis*. Allelic frequencies in each population are proportional to the area of the circle occupied by its symbol.

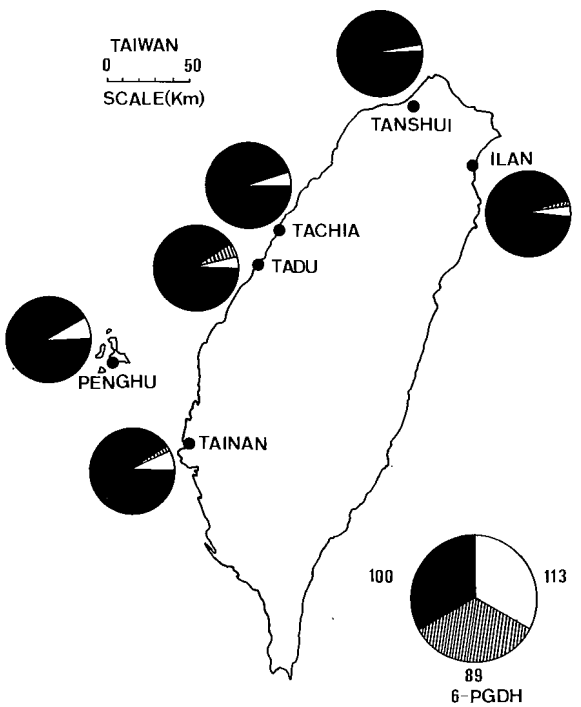

Fig. 3. Allelic frequencies at the *6-PGDH** locus in six populations of *Periophthalmus cantonensis*. Allelic frequencies in each population are proportional to the area of the circle occupied by its symbol.

Table 3. Comparison of Nei's (1978) genetic similarity coefficient (I, above diagonal) and genetic distance (D, below diagonal) between pairs of samples of *Periophthalmus cantonensis* from six locations

	TN	PH	IL	TS	TC	TU
TN	—	0.996	0.998	0.998	0.999	0.999
PH	0.004	—	0.993	0.998	0.993	0.993
IL	0.002	0.007	—	0.995	0.998	0.997
TS	0.002	0.002	0.005	—	0.995	0.994
TC	0.001	0.007	0.002	0.005	—	0.998
TU	0.001	0.007	0.003	0.006	0.002	—

morphism in all populations while the *SOD-2** locus presented three-banded heterozygotes.

Table 3 is Nei's (1978) genetic similarity (I) and genetic distance (D) for all pairwise combinations of the six populations of *Periophthalmus cantonensis*. High genetic similarities of 0.993-0.999 and low genetic distances of 0.001-0.007 are notable. Table 2 shows mean expected heterozygosities ranging from 0.0652 in the Tadu population to 0.0276 in the Penghu population; the observed heterozygosities proportion ranged from 2.02% in the Penghu population to 5.27% in the Ilan population. The polymorphic loci proportion based on the most common allele criterion, having a frequency level of 0.95, ranged from 0.0909 in the Tanshui population to 0.2727 in the Ilan population with the overall mean at 0.1742.

The result of Chi-square tests among populations for each polymorphic locus agrees with Hardy-Weinberg expectations as observed in most of isozyme loci examined. The Ilan population (Tables 4 and 5) having 7 loci present with significance at 0.01 level when compared to a maximum of 3 loci in other populations was an exception.

DISCUSSION

Shaklee et al. (1982) suggested that the mean genetic distance of 0.05 (0.002-0.065) and a mean genetic similarity of 0.97 are designated at the population level among fishes. Thus the overall mean similarity index of 0.996 (0.993-0.999) obtained from six mudskipper populations proves nearly identical genetic structure. It is curious that such a strong territorial amphibious species, demonstrates such close genetic similarity though geographically isolated. Genetic structure characteristics expressed in gene variants are usually correlated with the environmental variables including geographic isolation (Sakaizumi et al. 1983), temperature (Place and Powers 1979), salinity (Koehn and Siebenaller 1981), heavy metals (Nevo et al. 1983), and pesticides (Asztalos and Nemcsok 1985). Environmental variable significance was measured with the heterozygosity and polymorphic proportion data deviations from the Hardy-Weinberg equilibrium. Among the 19 loci tested, ten loci including *sAAT**, *CK-A**, *CK-B**, *G6PDH**, *LDH-B**, *MDH-A**, *MPI**, *6-PGDH**, *SDH** and *SOD-2** did not follow the Hardy-Weinberg equilibrium in all the populations examined (Table 5). The number of loci which deviated from the Hardy-Weinberg equilibrium varies with locality: 7 loci of Ilan, 4 of Tachia, 2 each for Penghu, Tadu and Tainan, and only one single locus for the Tanshui population. Though the Nei's genetic identity averaged in the present study is 0.996, the differences among populations is obvious in heterozygosities between the estuarine populations including Ilan, Tanshui, Tachia, Tadu, and Tainan ($\bar{H}$ = 0.056) and that of the offshore Penghu Island population (0.028). The observed heterozygosity of 0.0227 obtained at the *SDH** locus in Penghu population is much lower than those of the other 5 populations (0.3714-0.4783). It has the highest F_{st} values of 0.1103 at allele *70* and 0.0912 at allele *100* (Table 2). Quantitatively speaking, $F_{st} = 0$, indicates no genetic differentiation among subpopulations; $F_{st} <$ 0.05 indicates very slight differentiation which is by no means negligible; $F_{st} = 0.05$-0.15 indicates moderate differentiation; $F_{st} = 0.15$-0.25 indicates great differentiation and $F_{st} > 0.25$ indicates a very great differentiation (Wright 1978). The mean F_{st} differentiation among populations of 0.0235 obtained in this study is negligible. Of note is the role of the *SDH** locus in the Chinese mudskipper. Lin et al. (1969) obtained higher heterozygosity in wild populations than those of the domesticated goldfish at the *SDH** locus. A polluted environment selects for heterozygosity at a number of gene loci (Antonio and Ohno 1970). A similar situation is presently observed at the *SDH** locus in this study; the temperature difference among all habitats is minimum, the salinity conditions are typically estuarine in nature, and the status of the geographic isolation are similar among each population. The heavy metal content represents various degrees of pollution found within each habitat (Table 6). Minor gene variant difference among the 6 presumptive populations are probably related to the degree of heavy metal environmental impact sediment in the 6 habitats inspected (Table 6).

Table 4. The observed and expected genotype number to test chi-square for *Periophthalmus cantonensis* populations from Tainan (TN), Penghu (PH), Ilan (IL), Tanshui (TS), Tachia (TC), and Tadu (TU)

Locus	Genotype	TN		PH		IL		TS		TC		TU	
		obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
*sAAT**	129/129	—	0.014	—	—	—	0.005	—	0.032	2	0.134	—	0.007
	129/100	1	1.905	—	—	1	0.990	2	1.920	1	4.700	1	0.479
	100/100	68	67.082	45	45	49	49.005	29	29.048	43	41.166	35	34.999
*mAAT**	315/315	—	—	—	—	—	—	—	0.008	—	—	—	—
	315/100	—	—	—	—	—	—	1	0.976	—	—	—	—
	100/100	70	70	45	45	50	50	30	30.012	46	46	36	36
*ADH**	100/ 60	1	0.973	—	—	1	1.001	—	—	—	—	—	—
	100/100	69	69.023	45	45	45	44.993	31	31	46	46	36	36
	60/ 60	—	0.003	—	—	—	0.005	—	—	—	—	—	—
*CK-A**	125/125	—	—	—	—	—	—	—	—	—	—	—	0.065
	125/100	—	—	—	—	—	—	—	—	—	—	3	2.709
	125/112	—	—	—	—	—	—	—	—	—	—	—	0.086
	112/112	—	0.041	—	—	1	0.046	—	—	—	—	—	0.114
	112/100	3	2.942	—	—	1	2.884	—	—	—	—	4	3.591
	100/100	53	53.017	45	45	46	45.070	31	31	46	46	28	28.350
*CK-B**	100/100	70	70	45	45	49	48.020	31	31	46	46	36	36
	100/ 95	—	—	—	—	—	1.960	—	—	—	—	—	—
	95/ 95	—	—	—	—	1	0.020	—	—	—	—	—	—
*G-6PDH**	130/130	2	0.059	—	—	2	0.085	1	0.032	5	1.009	2	0.123
	130/100	—	3.942	—	—	—	3.863	—	1.921	3	11.002	—	3.780
	100/100	68	65.999	44	44	46	44.053	30	29.048	34	29.989	31	29.097
*GPI-A**	118/118	—	0.132	2	1.625	—	0.048	—	0.001	—	0.050	—	0.057
	118/100	6	5.236	13	13.338	3	2.719	1	0.918	3	2.541	8	6.807
	100/100	52	52.063	27	27.378	38	38.409	25	25.136	31	32.226	21	21.185
	100/ 80	9	9.639	3	2.106	6	5.438	3	2.808	12	10.010	6	5.238
	80/ 80	1	0.446	—	0.041	—	0.192	—	0.078	—	0.777	—	0.329
	118/ 80	—	0.485	—	0.513	—	0.193	—	0.051	—	0.395	1	0.849
*GPI-B**	200/200	—	0.003	—	—	—	0.022	—	—	—	—	—	0.007
	200/100	1	0.973	—	—	2	1.936	—	—	—	—	1	0.994
	100/100	69	69.023	45	45	43	43.042	29	29	46	46	35	34.999
*LDH-A**	65/ 65	—	0.003	—	—	—	—	—	—	—	0.006	—	—
	100/ 65	1	0.973	—	—	—	—	—	—	1	1.001	—	—
	100/100	69	69.023	45	45	50	50	31	31	45	44.993	36	36
*LDH-B**	60/ 60	—	0.015	—	—	1	0.125	—	0.008	—	—	—	—
	100/ 60	2	1.950	—	—	3	4.750	1	0.976	—	—	—	—
	100/100	64	64.035	45	45	46	45.125	30	30.016	46	46	36	36
*LDH-C**	96/ 96	—	0.005	—	—	—	0.005	—	0.008	—	0.022	—	—
	100/ 96	1	1.035	—	—	1	0.990	1	0.969	2	1.936	—	—
	100/100	57	56.961	38	38	49	49.005	28	28.022	43	43.042	36	36
*MDH-A**	100/100	69	69	45	45	49	49.005	31	31	46	46	36	36
	100/ 80	—	—	—	—	—	0.990	—	—	—	—	—	—
	80/ 80	—	—	—	—	1	0.005	—	—	—	—	—	—
*MDH-B**	125/125	—	0.003	—	—	—	—	—	0.009	—	—	—	—
	125/100	1	0.959	—	—	—	—	1	1.003	—	—	—	—
	100/100	68	68.037	45	45	50	50	29	28.989	44	44	35	35
*MPI**	104/ 86	—	0.021	—	—	—	0.180	—	0.017	—	—	—	—
	104/104	—	0.003	—	—	1	0.045	—	0.493	—	—	—	—
	104/100	1	0.938	—	—	1	2.730	1	0.952	—	—	—	—
	100/100	65	65.056	45	45	42	41.405	27	27.061	44	43.998	35	34.999
	100/ 86	3	2.948	—	—	6	5.460	1	0.952	2	1.979	1	0.994
	86/ 86	—	0.033	—	—	—	0.180	—	0.008	—	0.022	—	0.007

Table 4. Continued

Locus	Genotype	TN		PH		IL		TS		TC		TU	
		obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
*PGM-A**	116/100	1	0.959	—	—	—	—	1	0.950	—	—	—	—
	100/100	68	68.037	45	45	50	50	24	24.061	46	46	36	36
	100/ 94	—	—	—	—	—	—	1	0.950	—	—	—	—
	116/116	—	0.003	—	—	—	—	—	0.009	—	—	—	—
	116/ 94	—	—	—	—	—	—	—	0.019	—	—	—	—
	94/ 94	—	—	—	—	—	—	—	0.019	—	—	—	—
*PGM-B**	100/100	66	65.997	45	45	50	50	25	25.021	46	46	35	34.999
	100/ 75	3	2.969	—	—	—	—	1	0.969	—	—	1	0.994
	75/ 75	—	0.033	—	—	—	—	—	0.009	—	—	—	0.007
*6-PGDH**	113/113	3	0.363	2	0.274	—	0.045	—	0.008	1	0.085	—	0.063
	113/100	4	9.213	3	6.472	3	2.850	1	0.976	2	3.786	1	2.731
	100/100	61	58.478	40	38.254	45	45.125	30	30.016	43	42.129	32	29.355
	100/ 89	2	1.791	—	—	2	1.900	—	—	—	—	—	3.576
	113/ 89	—	0.139	—	—	—	0.060	—	—	—	—	2	0.166
	89/ 89	—	0.014	—	—	—	0.020	—	—	—	—	1	0.109
*SDH**	113/113	—	—	2	0.089	—	—	—	—	—	—	—	—
	113/100	—	—	—	3.734	—	—	—	—	—	—	—	—
	113/ 70	—	—	—	0.047	—	—	—	—	—	—	—	—
	100/100	31	31.355	41	39.127	22	22.445	16	16.116	19	19.555	15	13.195
	100/ 70	24	23.312	1	0.996	23	22.110	3	2.765	22	20.874	13	16.590
	70/ 70	4	4.333	—	0.060	5	5.445	—	0.118	5	5.571	7	5.215
*SOD-2**	136/136	—	0.016	—	—	3	0.245	—	—	3	0.194	—	—
	136/100	2	1.984	—	—	1	6.510	—	—	—	5.591	—	—
	100/100	61	61.000	45	45	46	43.245	31	31	43	40.214	36	36

Table 5. The observed and expected genotype number to test chi-square for *Periophthalmus cantonensis* populations from Tainan (TN), Penghu (PH), Ilan (IL), Tanshui (TS), Tachia (TC), and Tadu (TU)[1]

Locus	Locations						
	TN	PH	IL	TS	TC	TU	Total samples
*sAAT**	0.456	—	0.005	0.473	28.980**	0.516	30.430**
*mAAT**	—	—	—	0.009	—	—	0.009
*ADH**	0.004	—	0.005	—	—	—	0.009
*CK-A**	0.042	—	21.035**	—	—	0.347	21.424**
*CK-B**	—	—	50.000**	—	—	—	50.000**
*G6PDH**	68.008**	—	47.275**	31.530**	22.142**	32.547**	201.502**
*GPI-A**	1.458	1.033	0.524	0.151	1.748	2.824	7.738
*GPI-B**	0.004	—	0.024	—	—	0.007	0.035
*LDH-A**	0.004	—	—	—	0.006	—	0.010
*LDH-B**	0.016	—	6.787**	0.009	—	—	6.812**
*LDH-C**	0.006	—	0.005	0.009	0.024	—	0.044
*MDH-A**	—	—	198.975**	—	—	—	198.975**
*MDH-B**	0.005	—	—	0.009	—	—	0.014
*MPI**	0.062	—	21.784**	0.523	0.022	0.007	22.398**
*PGM-A**	0.005	—	—	0.042	—	—	0.047
*PGM-B**	0.033	—	—	0.010	—	0.007	0.050
*6-PGDH**	22.981**	12.815**	5.393	0.009	10.71*	32.517**	84.425**
*SDH**	0.050	44.963**	0.681	0.139	0.135	1.635	47.603**
*SOD-2**	0.016	—	35.819**	—	46.370**	—	82.205**

[1]*significant at $p < 0.05$;
**significant at $p < 0.01$.

Table 6. Heavy metals contained in the sediments from Tainan (TN), Penghu (PH), Ilan (IL), Tanshui (TS), Tachia (TC), and Tadu (TU)

Locations	Heavy metals (ppm)			
	Cu	Zn	Cd	Ni
TN	3.00 ± 0.13	9.87 ± 0.54	0.36 ± 0.20	2.13 ± 0.16
PH	0.35 ± 0.03	0.15 ± 0.05	0.26 ± 0.01	1.77 ± 0.01
IL	13.54 ± 0.20	23.91 ± 0.26	0.18 ± 0.02	3.70 ± 0.01
TS	9.25 ± 0.03	18.45 ± 0.07	0.11 ± 0.02	3.94 ± 0.01
TC	8.20 ± 0.01	20.47 ± 0.05	0.09 ± 0.03	3.87 ± 0.01
TU	12.46 ± 0.03	43.40 ± 0.42	0.18 ± 0.01	26.43 ± 2.62

Heavy metal detection method from Nelson et al. 1959.

A higher concentration of copper and zinc from the upper layer of soil at Tachia, Tadu, and Ilan estuaries coincides with higher expected heterozygosity (0.0636-0.0652, Table 2) in the three respective populations. However, the highly concentrated copper and zinc in the mangrove soil with a contradictory lower heterozygosity level of 0.0336 (Table 2) in the mudskipper living in the Tanshui estuary is an unusual indicator suggesting that the associated ovoviviparous *Kandelia candel* plays a role since *K. candel* can uptake a great amount of heavy metals from the surroundings (Chiu and Chou 1991). There are no mangrove forests in the Tachia, Tadu, and Ilan estuaries.

With the exception of Cd, the Penghu Island habitat has the lowest heavy metal sediment content in association with the lowest expected heterozygosity of 0.0276 in the mudskipper supporting a positive correlation between genetic variation and environmental variability.

The Chinese mudskipper from Tainan with the least influence of fresh water had the expected heterozygosity of 0.053 which is close to the 5% levels designated for teleosts (Wakeman and Ramsey 1988). Regression analysis between mean heterozygosity and Zn, Cu, Ni, Cd, resulted in correlation coefficients values (r) among populations of 0.708, 0.658, 0.446 and 0.179 respectively for Zn, Cu, Ni and Cd. This revealed that Zn and Cu ($r > 0.500$) have a significant positive correlation with population mean heterozygosity.

The high genetic similarity among these populations suggests they have the same origin though the population divergence mechanism is not yet clear. Genetic variation is linked with environmental heterozygosity (Wallis and Beardmore, 1984). Environmental factors such as heavy metal concentration may correlate with mudskipper mean heterozygosity. A decline in the survivorship with an increase in mudskipper heterozygosity are expected if human activities continue to impact rivers.

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臺灣產彈塗魚（鱸目，鰕虎科）之遺傳變異

張 穀 悌　　李 信 徹

總共有278尾的中國彈塗魚從臺灣六個地區包括淡水、宜蘭、台南、大甲、大肚及澎湖採集並藉由澱粉膠電泳法來估計其遺傳上分歧度之程度，14個同功酶中共得到二十二個基因座 (loci) 及四十七個等位基因 (alleles)，其Nei氏平均遺傳相似值爲0.996（其值介於0.993-0.999之間）代表具有高度相似的蛋白質結構，多型性基因(polymorphic loci)，在0.95水準範圍從0.0909到0.2727平均值爲0.1742，期望平均異型結合數(ex-mean heterozygosity)從0.028到0.065平均值爲0.051，而各族群之間的平均分化度的F_{st}值僅爲0.0235，結果顯示大多數的族群並不符合哈溫平衡，可能是由於某些因素例如土壤中的重金屬含量影響此結果。

關鍵詞：彈塗魚，同功酶多型性，遺傳變異與環境變化相關性。