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Genetic Dimorphism in *Pseudocaranx dentex* from Tosa Bay, Japan

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Genetic differentiation in *Pseudocaranx dentex* caught in Tosa Bay was studied by an electrophoretic survey of 28 isozyme and protein loci. Genetic dimorphism was found among samples and the allele substitution was observed at two loci (*SP-4** and *SDH**). The genetic distance between them was 0.095, falling within a range of differentiation at the species level. The number of vertebrae showed a clear difference between the two types, 25 vs. 24. These data suggest that an undescribed species might be included in *P. dentex* in Japan, and emphasize the need for a taxonomical review of this species.

Pseudocaranx dentex is a carangid fish distributed world-wide in tropical and warm temperate marine waters, except the east Pacific region. Three species, *P. dentex*, *P. wrighti* and *P. chilensis* have been reported in the genus.[1] From Japan, only *P. dentex* has been recorded, its identification being based on morphological characters.[2] The economic importance of this species is now growing in put-and-take fisheries and pen-net culture in Japan and genetic research is therefore needed to clarify its exact taxonomic status. Morphological difference was found in the number of vertebrae in *P. dentex* collected from Tosa Bay, and we call the sample with 25 vertebrae the A type and that with 24 vertebrae the B type. We conducted an electrophoretic analyses on samples of *P. dentex* from Tosa Bay and found two distinct genetic types. Our objective in this study is to clarify the genetic relationships between the two types.

Materials and Methods

Specimens used in this study (n=94) were obtained in spring, 1989 (n=46) and 1990 (n=48) in Tosa Bay. Fifteen of the specimens were caught by angling at the end of April, 1989 at Kutsu fishing port, Susaki city, central Tosa Bay, and the remaining specimens (n=79) consisted of fish captured from whole Tosa Bay, conserved together for seeds of the pen-net culture. All samples were stored at −20°C until use.

Isozyme and protein gene markers from the tissue of skeletal muscle, liver, and heart were detected by starch gel electrophoresis.[3,4] The analyzed enzymes, their presumed loci, tissue source, and buffer systems used are shown in Table 1. The terminology of isozymes and loci followed Shaklee *et al.*.[5] Allelic frequencies were calculated directly from the observed genotype. Due to the relatively small sample number, genetic differentiation was quantified by genetic distance using Nei's method.[6]

Three meristic characters were counted after fixing in 10% formalin solution: vertebrae, including the urostyle, dorsal fin soft rays, and anal fin soft rays. Verterbral number was counted from radiographs.

Results

Electrophoretic Analysis

Twenty-eight genetic loci presumed by 15 enzymes and one non-enzymatic protein were scored. Genetic variants were observed at seven loci, while no variant was scored in the remaining 21 loci. Allele frequencies are shown in Table 2. Chi-square tests were performed for the distribution of genotypes with the Hardy-Weinberg equillibrium. No statistically significant difference between observed and expected allelic frequencies calculated from genotypes was detected at all loci examined. Genetic interpretation of banding patterns of polymorphic loci are described below.

Sarcoplasmic protein (*SP-4*; Fig. 1A). Three alleles, **−100*, **−120* and **−150* were estimated at this locus. The A type was monomorphic and fixed for the **−100* allele, whereas the B type was polymorphic, characterized by showing

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Table 1. The list of enzymes and protein examined, locus detected, tissue assayed and buffer systems used

Enzyme and protein	No. of EC	Locus	Tissue	Buffer
Alcohol dehydrogenase (ADH)	1.1.1.1	*ADH**	Liver	C-APM
Aspartate aminotransferase (AAT)	2.6.1.1	*AAT-1**	Liver	C-APM
		*AAT-2**	Liver	C-APM
Estrase (EST)	3.1.1.3	*EST-1**	Liver	C-APM
		*EST-2**	Liver	C-APM
Fumarase (FM)	4.2.1.2	*FM**	Muscle	C-APM
Glucose phosphate isomerase (GPI)	5.3.1.9	*GPI-1**	Muscle	C-APM
		*GPI-2**	Heart	C-APM
α-Glycerophosphate dehydrogenase (αGPD)	1.1.1.8	*αGPD-1**	Liver	C-APM
		*αGPD-2**	Muscle	C-APM
Isocitrate dehydrogenase (IDH)	1.1.1.42	*IDHP-1**	Liver	C-APM
		*IDHP-2**	Muscle	C-APM
Lactate dehydrogenase (LDH)	1.1.1.27	*LDH-1**	Muscle	C-APM
		*LDH-2**	Muscle	C-APM
Malate dehydrogenase (MDH)	1.1.1.37	*MDH-1**	Muscle	C-APM
		*MDH-2**	Muscle	C-APM
		*MDH-3**	Muscle	C-APH
Malic enzyme (ME)	1.1.1.40	*ME**	Liver	C-APM
Mannose phosphate isomerase (MPI)	5.3.1.8	*MPI**	Liver	C-APM
Phosphoglucomutase (PGM)	2.7.5.1	*PGM**	Muscle	C-APM
6-Phosphogluconate dehydrogenase (G6PDH)	1.1.1.44	*G6PDH**	Liver	C-APM
Sorbitol dehydrogenase (SDH)	1.1.1.14	*SDH**	Liver	T-C
Superoxide dismutase (SOD)	1.15.1.1	*SOD**	Liver	C-APM
Sarcoplasmic protein (SP)		*SP-1**	Muscle	C-APM
		*SP-2**	Muscle	C-APM
		*SP-3**	Muscle	C-APM
		*SP-4**	Muscle	C-APM
		*SP-5**	Muscle	C-APM

C-APM (citric acid-aminoprophyl morpholine, pH 6.0), gel density of 12%, T-C (tris-citric acid, pH 8.0) gel density of 12%.

*-120 and *-150 alleles and the gene frequency for *-150 was predominant. These two types shared no allele.

Sorbitol dehydrogenase (*SDH*; Fig. 1B). One locus was scored in the liver extract. Four alleles, **100*, **80*, **0* and *-20 were estimated at this locus. Both A and B types were polymorphic and shared no alleles at this locus. The A type was characterized by having the **100* and **0* alleles, whereas the B type was characterized by having the **80* and *-20 alleles.

Mannose phosphate isomerase (*MPI*) One *MPI* locus was scored in the liver extract. Three alleles, designated as **100*, **90* and **80* were estimated. The A type was monomorphic, fixed for the **100* allele. The B type was polymorphic, characterized by showing the three alleles, and the gene frequency for **90* was predominant.

Isocitrate dehydrogenase (*IDHP*-1). Three alleles, **120*, **100* and **70* were estimated at this locus. Both A and B types were polymorphic but the gene frequency for **100* was predominant.

Phosphoglucomutase (*PGM*). One *PGM* locus was scored in the muscle extract. Two alleles, *-80 and *-100 were estimated at this locus. The A type was monomorphic, fixed for the *-100 allele, whereas the B type was polymorphic and the gene frequency for *-100 was predominant.

Fumarase (*FM*). One locus was scored in the muscle extract. Three alleles, **120*, **100* and **80* were estimated at this locus. Both A and B types were polymorphic and shared the **100* allele which was predominant.

6-Phosphogluconate dehydrogenase (*G6PDH*). One locus was scored in the liver extract. Two alleles, designated as **100* and **80* were estimated. Both A and B types were polymorphic but the

Table 2. Allele frequencies for isozyme and sarcoplasmic protein loci from *P. dentex*

Locus	Allele	Type A		Type B	
		(89)	(90)	(89)	(90)
*ADH**	*−100	1.000	1.000	1.000	1.000
*AAT-1**	* 100	1.000	1.000	1.000	1.000
*AAT-2**	* 100	1.000	1.000	1.000	1.000
*EST-1**	* 100	1.000	1.000	1.000	1.000
*EST-2**	* 100	1.000	1.000	1.000	1.000
*FM**	* 120	—	0.042	—	—
	* 100	1.000	0.958	1.000	0.792
	* 80	—	—	—	0.208
*GPI-1**	* 100	1.000	1.000	1.000	1.000
*GPI-2**	* 100	1.000	1.000	1.000	1.000
*αGPD-1**	* 100	1.000	1.000	1.000	1.000
*αGPD-2**	* 100	1.000	1.000	1.000	1.000
*IDHP-1**	* 120	—	0.021	—	—
	* 100	1.000	0.979	0.980	0.979
	* 70	—	—	0.020	0.021
*IDHP-2**	* 100	1.000	1.000	1.000	1.000
*LDH-1**	* 100	1.000	1.000	1.000	1.000
*LDH-2**	* 100	1.000	1.000	1.000	1.000
*MDH-1**	* 100	1.000	1.000	1.000	1.000
*MDH-2**	* 100	1.000	1.000	1.000	1.000
*MDH-3**	* 100	1.000	1.000	1.000	1.000
*ME**	* 100	1.000	1.000	1.000	1.000
*MPI**	* 100	1.000	1.000	0.170	0.313
	* 90	—	—	0.780	0.625
	* 80	—	—	0.050	0.062
*PGM**	* −80	—	—	0.020	—
	*−100	1.000	1.000	0.980	1.000
*G6PDH**	* 100	0.940	0.979	0.970	0.979
	* 80	0.060	0.021	0.030	0.021
*SDH**	* 100	0.880	0.979	—	—
	* 80	—	—	0.980	1.000
	* 0	0.120	0.021	—	—
	* −20	—	—	0.020	—
*SOD**	* 100	1.000	1.000	1.000	1.000
*SP-1**	* 100	1.000	1.000	1.000	1.000
*SP-2**	* 100	1.000	1.000	1.000	1.000
*SP-3**	*−100	1.000	1.000	1.000	1.000
*SP-4**	*−100	1.000	1.000	—	—
	*−120	—	—	—	0.021
	*−150	—	—	1.000	0.979
*SP-5**	*−100	1.000	1.000	1.000	1.000
		N=16	24	30	24
No. of loci		28	28	28	28
No. of P+P*		2	4	5	5
No. of P*		2	0	1	2
Proportion of P+P*		0.071	0.143	0.176	0.179

P*: Criterion for polymorphism is lower than 0.95 in major allele frequency. P. Polymorphic.

gene frequency for **100* was predominant.

In the 15 specimens from Kutsu fishing port, 6 A and 9 B type specimens were included. In the 79 specimens from whole Tosa Bay, 34 A and 45 B type specimens were found. The mean and SD of the fork length of A and B types specimens was 12.78±1.42 cm and 14.43±1.51 cm, respectively.

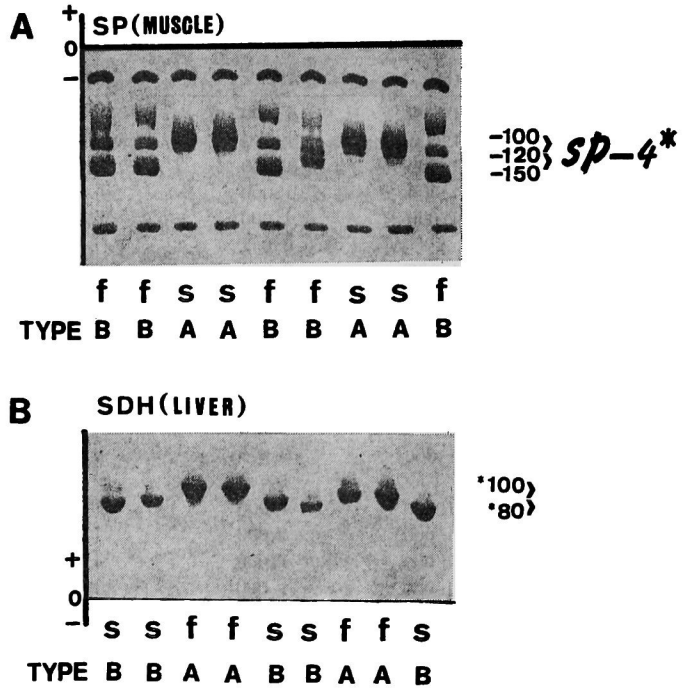

Fig. 1. Examples of electropherograms of SP and SDH. s: slow, f: fast.

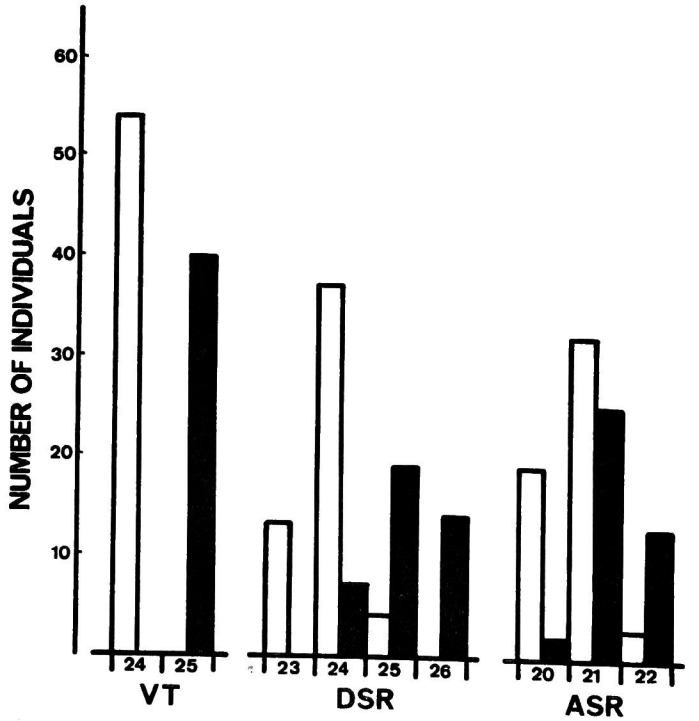

Fig. 2. Frequency distributions of vertebral number (VT), dorsal soft ray number (DSR) and anal soft ray number (ASR). Open bar shows B type and solid bar A type.

No hybrid specimen was detected during the study.

Genetic Distance

Estimates of genetic distance between the two types were computed according to Nei's method[6] on the basis of the small number of individuals. Results showed a genetic distance of 0.095 between the two types.

Morphological Comparison

The number of vertebrae, dorsal soft rays, and anal soft rays was counted (Fig. 2). Among these three characters, only the vertebral number showed a clear difference between the two types. All samples of the A type had 25 vertebrae, while all B type samples had 24 vertebrae. No clear difference was detected in the number of dorsal and anal soft rays, but the samples of B type tended to have fewer soft rays. In juveniles and youngs of the B type, the 12 vertical stripes on the lateral side appeared to be more distinct than those of juveniles and youngs of A type. The A type was smaller than the B type in body length.

Discussion

Among the genera in the subfamily Caranginae, the genus *Pseudocaranx* is genetically most closely related to the genus *Kaiwarinus* (D=0.41).[7] Therefore, both A and B types can be regarded as species belonging to the genus *Pseudocaranx*. Clear allele substitutions between the two types were observed at tow loci, *i. e. SP-4* and *SDH*. In addition, the genetic distance of 0.095 found between the two types in this study falls within the range of values reported between species of marine teleost fishes.[8] In the genus *Trachurus*, also the member of Caranginae, some interspecific genetic distance were smaller than 0.1.[9] In bottom-living sparids and sciaenids, the average genetic distance between congeneric species tends to be small (D=0.115 and 0.092, respectively).[10,11] These two types are most likely discrete species.

P. dentex from waters adjacent to Japan has 25 vertebrae.[12,13] Similarly, the vertebral number of this species from temperate Australasian waters is mainly 25.[14] *P. dentex* and *P. wrighti* are distributed in the west Pacific,[1] but the latter species always has 24 vertebrae.[15] Therefore, the type A detected by electrophoretic analyses in this study, showing 25 vertebrae, is almost certainly *P. dentex*.

However, the B type is not *P. wrighti*, the described species having 24 vertebrae. *P. wrighti* differs from B type in having a maxilla of which the exposed part is scaled,*1 whereas B type has no scales on the maxilla*2. Is the B type the third described species of this genus, *P. chilensis?* Almost certainly not, because *P. chilensis* is endemic to the east Pacific.[1] Together, these facts strongly suggest that the two types in this study are discrete species in the genus *Pseudocaranx* and that the B type is a new species. A taxonomical review is strongly needed for this species.

At Kutsu fishing port, specimens of the two types were collected together at the same place in the same morning, which suggests that the two types make a mixed school in the juvenile and young stages. However, as yet, no hybrid has been found, suggesting that the breeding sites and/or season of the two types are subltly but clearly different from each other.

In this study, the number of specimens examined of the A type was smaller than that of the B type. However, this ratio does not coincide with that of the natural populations, because we selected samples which had more or less distinct vertical bands on the flanks (B type) from the fish conserved for aquaculture. The population density of the B type, therefore, does not seem to be negligible.

This study clearly demonstrated that electrophoretically detectable isozymic alleles are still very useful markers to investigate the taxonomic validity of common fish such as *P. dentex*.

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*1 Gushiken: pers. comm.

*2 K. Yamaoka: unpublished.

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