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Biochemical Polymorphism in Yellow Catfish, *Mystus nemurus* (C&V), from Thailand

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Yellow catfish, Mystus nemurus *(Cuv. & Val.), is becoming one of the major freshwater species farmed by aquaculturists in Southeast Asia. It was of interest to examine levels of genetic subpopulation differentiation among samples of this species obtained from parts of its range, as well as to compare the genetics of wild and hatchery-bred fish. Horizontal starch gel electrophoresis and histochemical staining techniques were used to examine genetic variation within and among eight wild and one hatchery populations of* M. nemurus *from northern, northeastern, central and southern Thailand. Four tissues (heart, liver, kidney, and muscle) from individual specimens were used to analyze variations at 23 protein-coding loci. Fifteen of the 23 loci examined (65.22%), namely,* ACP*, AAT-1*, EST-1*, EST-2*, GPI*, IDH-1*, IDH-2*, MDH-1*, MDH-2*, MDH-3*, ME*, PGM*, 6PGD*, SOD*, *and* HB*, *were polymorphic at the 0.95 level. Observed heterozygosities ranged from 0.041 to 0.111, with an average of 0.068 ± 0.028. Genetic distances ranged from 0.005 to 0.164. The greatest genetic distance was found between the Chainat and the Suratthani populations (0.164), a level indicative of subspecific differentiation in* M. nemurus *from within Thailand.*

KEY WORDS: *Mystus nemurus;* yellow catfish; genetic variation; electrophoresis; allozymes.

INTRODUCTION

Yellow catfish, *Mystus nemurus* (Cuvier and Valenciennes), is one of the most important freshwater fish cultured in Southeast Asia because of its palatability. In

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Thailand, Malaysia, and other Southeast Asian countries, the fish is typically found in rivers, swamps, lakes, and other bodies of water (Amatyakul *et al.,* 1995; Inger and Chin, 1962; Mohsin and Ambak, 1983; Wongrat and Krudphan, 1994; Smith, 1945). In Thailand, it is found throughout the country and it is highly preferred by fish farmers. Mass production of *M. nemurus* fries was achieved by the Thai Fisheries Department using artificial breeding (Amatyakul *et al.,* 1995) and fries are typically distributed to fish farmers for stocking in ponds and natural and public water bodies.

Loss of genetic variation (heterozygosity) has been shown in several cultured stocks to be associated with the deterioration of important production characteristics such as fecundity, survival, and growth (Crozier and Moffett, 1989; Stahl, 1987). Since there is no published work available on genetic variation in *M. nemurus* in Thailand, future exploitation of this species for intensive aquaculture programs should be preceded by studies providing information on the genetic relationships within and among its populations. Among the available genetic markers, allozymes have been shown to be powerful enough for studying genetic variation at intraspecific and interspecific levels in freshwater fish (Ward and Grewe, 1995). In this study, samples from eight wild populations representing the northern, northeastern, central, and southern parts of Thailand and one hatchery stock of *M. nemurus* were analyzed using horizontal starch gel electrophoresis followed by histochemical staining to estimate the genetic variation within and among the populations.

MATERIALS AND METHODS

Specimens of *Mystus nemurus* ranging from 10.5 to 41.0 cm in length and 7.0 to 781.3 g in weight were collected from eight locations in Thailand as shown in Fig 1 and Table I. A hatchery stock was obtained from the hatchery operated by the Suratthani Inland Fisheries Development Center (SIFDC), in southern Thailand. This hatchery was established in 1993 and mass produces fries for distribution to fish farmers and for stocking in natural and man-made bodies of water. Live fish were transported from the local areas to the nearest fisheries station for tissue collection. Heart, liver, kidney, and flank muscle tissues of each fish were collected and then stored at −80°C until the electrophoretic analysis.

Tissue homogenates were analyzed by horizontal starch gel electrophoresis according to the method of Taniguchi and Suguma (1990). The enzyme systems (Table II) were examined using an acetic acid–aminopropylmorpholine (C-APM) (pH 7.0) buffer. Staining procedures were those of Shaw and Prasad (1970). Sarcoplasmic protein and haemoglobin were stained with 0.1% amido black (7%

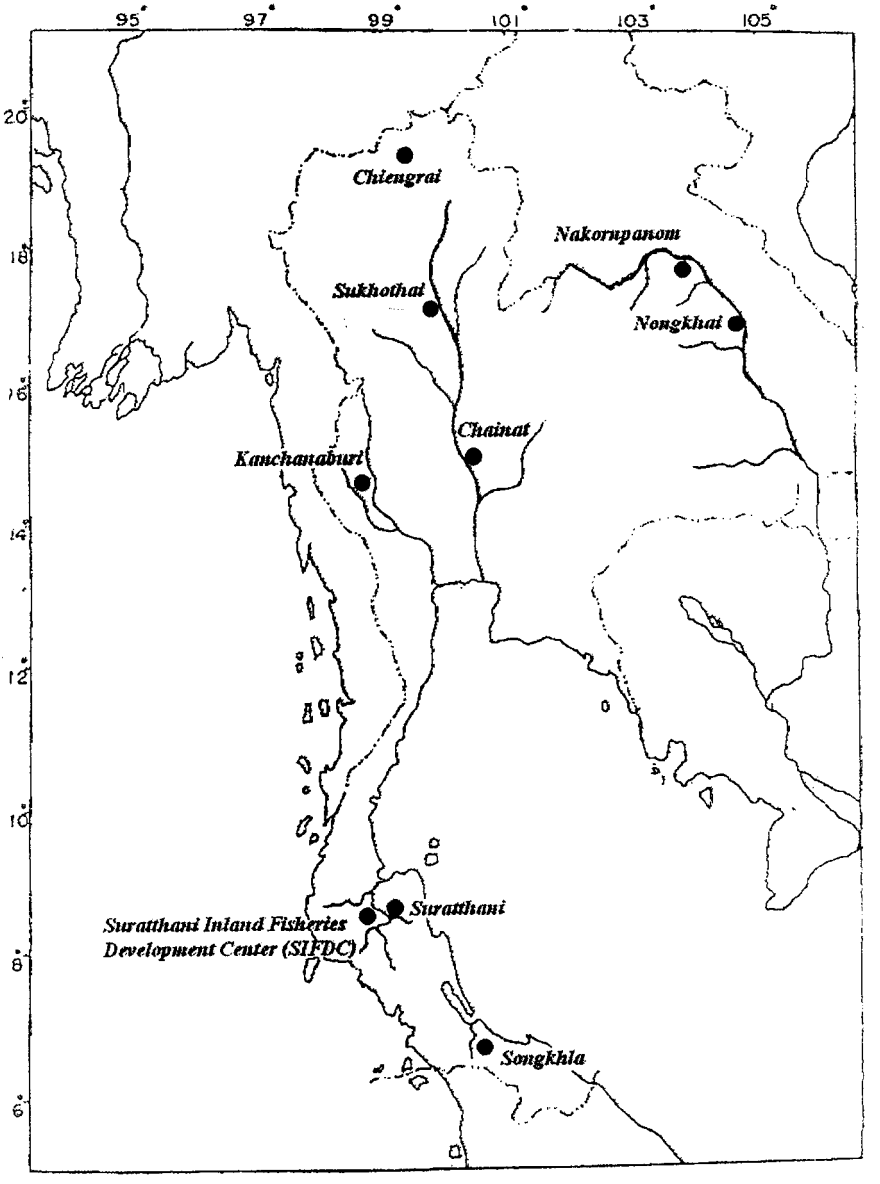

Fig. 1. Sampling locations of *Mystus nemurus* in Thailand.

Table I. Number, Total Length (cm), and Body Weight (g) of Yellow Catfish, *M. nemurus*, from Different Localities in Thailand

Location	Number of fish	Total length (cm)			Body weight (g)		
		Min.	Max.	Average	Min.	Max.	Average
Northern region							
(1) Chiengrai	30	13.2	20.7	16.00 ± 1.68	15.0	55.0	25.20 ± 8.39
(2) Sukhothai	30	21.1	26.6	24.10 ± 1.39	60.0	135.0	93.10 ± 19.13
Central region							
(3) Chainat	30	15.5	24.3	19.50 ± 2.01	35.0	115.0	68.10 ± 19.12
(4) Kanchanaburi	30	22.8	39.0	27.70 ± 3.36	150.0	365.0	230.20 ± 60.72
Northeastern region							
(5) Nongkhai	47	12.2	35.5	21.00 ± 4.85	13.0	306.0	75.90 ± 52.25
(6) Nakornpanom	46	10.5	25.5	16.20 ± 4.13	7.0	115.0	37.40 ± 28.96
Southern region							
(7) Suratthani	50	20.2	41.0	29.40 ± 4.74	68.9	781.3	214.20 ± 132.92
(8) Songkhla	50	21.1	39.5	27.20 ± 3.52	58.6	444.5	140.40 ± 64.69
Hatchery							
(9) Suratthani Inland Fisheries Development Center (SIFDC)	50	20.5	31.5	26.60 ± 2.57	66.5	250.7	144.30 ± 37.31

acetate). Fish loci nomenclature followed the conventions recommended by Shaklee *et al.* (1990). When enzymes were coded by multiple loci, loci were designated numerically according to their migration relative to the anode. The most common allele was designated 100, and other alleles were given numbers according to the mobility of their protein products relative to the mobility of allele 100. A locus was defined as polymorphic when the frequency of the most common allele was less than or equal to 0.95.

Data from individual samples in each population were used for the estimation of allele frequencies, heterozygosity, genetic distance (*D*), and variation distribution. Genetic distances were calculated using the formula proposed by Nei (1978), and a dendrogram was constructed by UPGMA clustering. Variation distribution was calculated following *F* statistics (Wright, 1978) and chi-square test was performed for conformity of genotypic frequencies to Hardy–Weinberg expectations. All analyses were performed using the BIOSYS-1 program (Swofford and Selander, 1989).

RESULTS AND DISCUSSION

Among the 23 loci detected (Table II), 15 loci (65.22%), namely, *ACP**, *AAT-1**, *EST-1**, *EST-2**, *GPI**, *IDH-1**, *IDH-2**, *MDH-1**, *MDH-2**, *MDH-3**, *ME**,

Table II. Enzymes, Sarcoplasmic Protein, and Hemoglobin Examined, Loci Identified and Tissue Specificity Observed in *M. nemurus*

Enzymes and proteins	Locus	Tissue specificity[a]
Acid phosphatase	*ACP**	L, K
Alcohol dehydrogenase	*ADH**	L, K
α-Glycerophosphate dehydrogenase	*α-GPD**	L, K, M
Aspartate aminotransferase	*AAT-1**	H, L, K, M
	*AAT-2**	H, L, K, M
Esterase	*EST-1**	H, L, K, M
	*EST-2**	H, L, K, M
Glucose phosphate isomerase	*GPI**	L
Isocitrate dehydrogenase	*IDH-1**	H, L, K
	*IDH-2**	H, K
Lactate dehydrogenase	*LDH-1**	H, L, K, M
	*LDH-2**	H, L, K, M
Malate dehydrogenase	*MDH-1**	H, L, K, M
	*MDH-2**	H, L, K, M
	*MDH-3**	H, L
Malic enzyme	*ME**	H, L, M
Octanol dehydrogenase	*ODH-1**	H, L, K
	*ODH-2**	L, M
Phosphoglucomutase	*PGM**	H, L, K, M
6-Phosphogluconate dehydrogenase	*6-PGD**	H, L, K
Superoxide dismutase	*SOD**	H, K
Sarcoplasmic protein	*SP**	M
Haemoglobin	*HB**	H, L, K

[a]H, heart; L, liver; K, kidney; M, muscle.

PGM, 6PGD*, SOD*,* and *HB*,* were polymorphic using the 0.95 criterion of polymorphism. The allele frequencies for polymorphic loci are presented in Table III. The average number of allele per locus ranged from 1.13 to 1.52 (Table IV).

There was no significant deviation from Hardy–Weinberg equilibrium in any population ($P < 0.05$), with F_{st} (fixation index) and F_{is} (inbreeding coefficient) values of 0.42 and 0.08, respectively. The highest heterozygosity was found in the hatchery population (0.111 ± 0.036) and the lowest in the Sukhothai population (0.041 ± 0.023). This is uncommon and unexpected and was probably due to the fact that the hatchery population broodstocks used at the SIFDC, southern Thailand, were regularly renewed from the wild by samples collected from various localities. The same situation was experienced in the Malaysian stocks (Daud *et al.,* 1989; Siraj *et al.,* 1998; Tay, 1997), with a similar trend of high heterozygosity values in the hatchery populations and the lowest in the natural populations.

The genetic distance of the *M. nemurus* populations in Nongkhai to that in Nakornpanom is small (0.005) (Table V), presumably because both areas are connected by the Maekhong River, thus, migration and mixing of populations

Table III. Allele Frequencies of Polymorphic Isozyme and Protein Loci (at 0.95) in *M. nemurus* (Population Numbers Correspond to Those in Table I)

		Population sampled								
Locus	Allele	1	2	3	4	5	6	7	8	9
*ACP**	−40	0.767	1.000	0.633	0.933	1.000	1.000	0.170	0.860	0.620
	−100	0.233	0.000	0.367	0.067	0.000	0.000	0.830	0.140	0.380
*AAT-1**	120	0.517	0.283	0.000	0.467	0.000	0.000	0.000	0.000	0.000
	100	0.483	0.717	1.000	0.533	1.000	1.000	1.000	1.000	1.000
*EST-1**	100	1.000	1.000	1.000	0.500	1.000	1.000	1.000	0.980	1.000
	80	0.000	0.000	0.000	0.500	0.000	0.000	0.000	0.020	0.000
*EST-2**	100	1.000	1.000	1.000	1.000	0.915	0.880	0.910	0.980	0.930
	80	0.000	0.000	0.000	0.000	0.085	0.120	0.090	0.020	0.070
*GPI**	100	0.000	0.000	0.000	0.017	0.000	0.000	0.990	0.590	0.750
	80	1.000	1.000	1.000	0.983	1.000	1.000	0.010	0.410	0.250
*IDH-1**	120	0.000	0.000	0.000	0.000	0.064	0.043	0.450	0.500	0.240
	100	1.000	1.000	1.000	1.000	0.936	0.957	0.550	0.500	0.760
*IDH-2**	120	0.583	1.000	1.000	1.000	1.000	1.000	0.600	1.000	1.000
	100	0.417	0.000	0.000	0.000	0.000	0.000	0.400	0.000	0.000
*MDH-1**	100	0.867	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	80	0.133	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*MDH-2**	100	1.000	1.000	1.000	1.000	0.957	0.870	1.000	1.000	1.000
	80	0.000	0.000	0.000	0.000	0.043	0.130	0.000	0.000	0.000
*MDH-3**	−100	1.000	1.000	1.000	1.000	1.000	1.000	0.930	1.000	1.000
	−150	0.000	0.000	0.000	0.000	0.000	0.000	0.070	0.000	0.000
*ME**	120	0.000	0.000	0.217	0.033	0.033	0.000	0.100	0.190	0.560
	100	1.000	1.000	0.783	0.967	0.967	1.000	0.900	0.740	0.440
	80	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.070	0.000
*PGM**	120	1.000	0.867	0.083	0.933	0.085	0.033	0.220	0.000	0.170
	100	0.000	0.133	0.917	0.067	0.915	0.957	0.780	1.000	0.820
	80	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.010
*6-PGD**	120	0.000	0.117	0.000	0.033	0.745	0.413	0.094	0.070	0.050
	100	1.000	0.883	0.949	0.950	0.255	0.587	0.844	0.920	0.890
	80	0.000	0.000	0.051	0.017	0.000	0.000	0.063	0.010	0.060
*SOD**	80	0.000	0.000	0.000	0.000	0.000	0.000	0.310	0.000	0.000
	100	1.000	1.000	1.000	1.000	1.000	1.000	0.690	1.000	1.000
*HB**	−80	0.000	0.000	0.000	0.000	0.085	0.098	0.420	0.140	0.240
	−100	1.000	1.000	1.000	1.000	0.915	0.902	0.580	0.860	0.760

may occur. The highest genetic distance (0.164) was found between the Chainat and the Suratthani populations, and this is supported by morphometric data on characters such as body depth, dorsal fin height, and anal fin height, which were significantly different ($P < 0.05$) between the two populations.

The UPGMA dendrogram (Fig. 2) depicts the genetic relationships among populations of *M. nemurus*. The dendrogram from Nei's genetic distances clearly indicates that the *M. nemurus* samples are grouped into three clusters. Samples from northern and central Thailand sharing the same drainage form one cluster,

Table IV. Genetic Variability at 23 Loci in All Populations (Standard Error in Parentheses) of *M. nemurus* in Thailand

Population	Rare allele	Mean number of alleles per locus	Percentage of loci polymorphic[a]	Mean heterozygosity	
				Direct count (Ho)	Hardy–Weinberg expected (He)[b]
(1) Chiengrai	0	1.17 (0.08)	17.39	0.055 (0.027)	0.070 (0.034)
(2) Sukhothai	0	1.13 (0.07)	13.04	0.041 (0.023)	0.037 (0.022)
(3) Kanchanaburi	0	1.17 (0.08)	17.39	0.048 (0.029)	0.047 (0.025)
(4) Chainat	2	1.35 (0.12)	21.74	0.067 (0.032)	0.064 (0.030)
(5) Nongkhai	2	1.30 (0.10)	21.74	0.044 (0.016)	0.049 (0.020)
(6) Nakornpanom	2	1.30 (0.12)	17.39	0.057 (0.027)	0.056 (0.025)
(7) Suratthani	1	1.52 (0.12)	43.48	0.102 (0.027)	0.144 (0.040)
(8) Songkhla	2	1.43 (0.14)	26.09	0.089 (0.034)	0.092 (0.035)
(9) Hatchery	0	1.43 (0.14)	34.78	0.111 (0.036)	0.119 (0.038)
Mean	1.00	1.31 (0.11)	23.67	0.068 (0.028)	0.075 (0.030)

[a]A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
[b]Unbiased estimate (Nei, 1978).

whereas *M. nemurus* samples from northeastern Thailand originating from the northeastern river system form another. The samples from southern Thailand comprise a third cluster, except for the Kanchanaburi sample, which clusters with the Nakornpanom and Nongkhai samples. The low estimate of genetic distance between the Suratthani Province and the wild Songkhla samples indicates that the broodstocks used at the Suratthani hatchery probably originated from Songkhla province, and these hatchery fish were then used in the restocking of fish in ponds and natural and public water bodies by commercial cage culture operators and/or the Department of Fisheries, Thailand.

Table V. Nei's (1978) Genetic Distances Among Pairs of Populations of *M. nemurus* in Thailand

Population	1	2	3	4	5	6	7	8	9
(1) Chiengrai	—	0.014	0.064	0.021	0.092	0.079	0.141	0.106	0.108
(2) Sukhothai		—	0.040	0.013	0.051	0.041	0.142	0.072	0.082
(3) Kanchanaburi			—	0.061	0.032	0.017	0.092	0.031	0.037
(4) Chainat				—	0.081	0.069	0.164	0.096	0.103
(5) Nongkhai					—	0.005	0.131	0.050	0.072
(6) Nakornpanom						—	0.118	0.036	0.059
(7) Suratthani							—	0.052	0.040
(8) Songkhla								—	0.014
(9) Hatchery									—

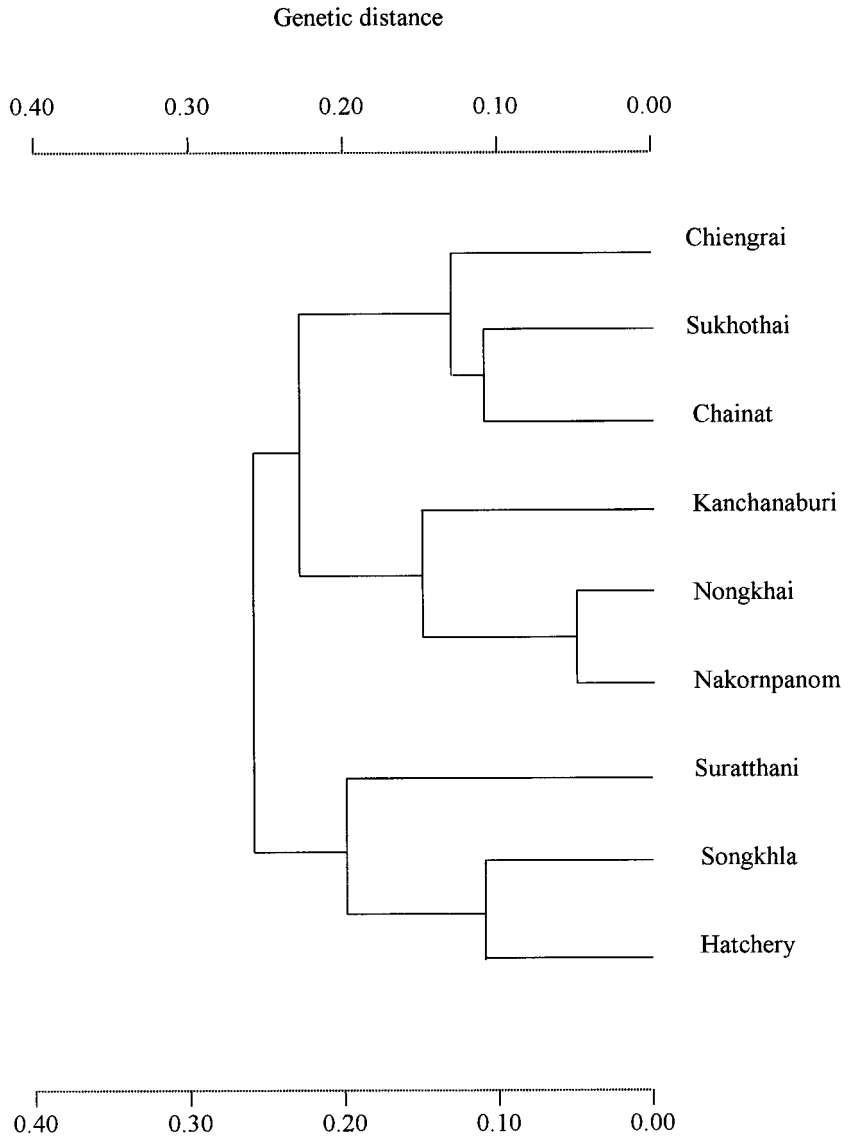

Fig. 2. UPGMA dendrogram constructed with Nei's (1978) genetic distances among populations of *M. nemurus* in Thailand.

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