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Liver Esterase Polymorphisms in Sepat Siam (*Trichogaster pectoralis*)

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RINGKASAN

Esterase D dan esterase am (yang menggunakan α- atau β-napthyl asetat atau kedua-dua sekali sebagai substrat) telah dikaji dalam ikan sawah padi, Trichogaster pectoralis. *Varian-varian telah dijumpai untuk enzim-enzim tersebut. Hipotesis telah dicadangkan bahawa fenotaip esterase D disebabkan oleh dua alel kodominan disatu lokus autosom dan empat lokus terlibat didalam kawalan fenotaip esterase am.*

SUMMARY

Esterase D and general esterases (which use α- or β-naphthyl acetate as substrates) were investigated electrophoretically in the paddy field fish, Trichogaster pectoralis. *Variants were observed for these enzymes. It is hypothesized that esterase D phenotypes are due to two codominant alleles at an autosomal locus, and that four loci are involved in the control of the general esterases.*

INTRODUCTION

Trichogaster pectoralis (Regan) is a dark leaf-like appearing Labyrinth fish that can grow up to 20 cm. in length. It is native to Thailand and Cambodia. (Smith, 1945). It was introduced into the Krian district, Perak, Peninsular Malaysia in the 1920's and since then it has multiplied rapidly and established itself in paddy areas, ponds and drains all over the country (Soong, 1948). It has also been introduced into Sarawak and the Philippines (Lowe-McConnel, 1975). It is an important economic species among the paddy field fishes in this country and thus acts as useful source of additional income to the paddy farmers. The fish is either sold alive or in salted form (Soong, 1948). Its local name in Malaysia is "Sepat Siam" while in Thailand it is known as "pla salid" or "pla bai mai". Tan *et al.* (1973) estimated that 72% of the total catch of paddy field fishes in the Krian district was "Sepat Siam". The other fishes were "Keli", *Clarias macrocephalus,* (20%), "Aruan", *Ophiocephalus striatus,* (6%) and "Puyu", *Anabas testudineus,* (2%).

Fish from padi fields are the most important source of fresh water fish in Malaysia. However, unlike the well developed practice of fish culture in, for example, Indonesia, Japan and Taiwan, where flooded padi fields are stocked with fish which are then managed, no such effort is made here. The wild stock fish which make their way into the flooded padi fields are merely restrained and harvested (Tan *et al.,* 1973). Unfortunately, with the introduction of the double cropping pattern of paddy cultivation, accompanied by the greater and wider use of insecticides, a steady decline in fish productivity from paddy areas was noted (Tan *et al.,* 1973). Also, with the rising prices of marine fish in this country in recent years, it has now become increasingly necessary to culture fresh water fish in ponds on a commercial scale, so that the protein intake of the local population will not be adversely affected.

At present, there is very little information available on the general biology of paddy field fish. Hence it is imperative that research be done on them. This study on the esterases of the "Sepat Siam" is an attempt to provide some

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basic information on the biochemical genetic variability present in our local population of this fish. Genetic data are needed as an aid in the management of fish populations, as well as in the study of fish evolution and population genetics (Allendorf and Utter, 1978).

MATERIALS AND METHODS

Fish were caught from two ponds and drains at the Universiti Pertanian Malaysia farm at the Serdang campus. The two ponds were about 300 meters apart but were connected to one another by a drainage system which was also joined to the paddy fields of the campus farm. Only adult fish of at least 10cm. length (from the tip of the snout to the starting point of the caudal fin) were sampled. Such fish are of reproductive age and may be considered adults. Sampling was done from July 1979 to January 1980. The fish were killed by a blow on the head, blotted dry and then dissected. The liver was removed and cut into pieces and a volume of 0.1 M Tris-HCl extraction buffer (pH 7.0) equal to the volume of the liver used was added (Buth, 1979). Mechanical homogenization was done in a homogeniser placed in an ice bucket and the sample was then centrifuged at 3,000 rpm for 15 minutes. The clear supernatant was imbibed onto a Whatman 1 filter paper strip and used for horizontal electrophoresis.

Esterase D was typed using a bridge buffer of 440mM boric acid, 40mM LiOH (pH=7.2) and a gel buffer of 13.5mM tris, 3.6mM citric acid, 4.4mM boric acid and 0.4mM LiOH, pH=7.2 (Hopkinson *et al.*, 1973). It was run by using horizontal starch gel electrophoresis at 150V for 15 hours at 4°C. 4-methyl umbelliferyl acetate was used as the substrate for staining.

General esterases were typed following the method of Brewbaker *et al.*, (1968), using a 0.1M boric acid, 0.036M LiOH bridge buffer(pH=8.3) and a 0.064M tris, 0.0086M citric acid gel buffer (pH=8.7). The 5% polyacrylamide gels were pre-run at 300V for 3 hours before use. The separation run was at 100V for 15 hours at 4°C. The gels were stained using α-napthyl acetate or β-napthyl acetate as substrates (Brewbaker *et al.*, 1968).

RESULTS AND DISCUSSION

Eighty-five samples of liver extracts were typed for esterase D (ESD). The electrophoretic patterns observed are presented in Figure 1 and the results in Table 1. There are altogether three types of phenotypes; we named them ESD SS, ESD FF and ESD FS. The most common phenotype, ESD SS had a single anodal migrating band and occurred in 71% of the samples, 27 females and 33 males. The second phenotype, ESD FF also had a single band, but it migrated faster toward the anode than the ESD SS band. Both the two individuals with this phenotype were females. The third phenotype, ESD FS, occurred in 27% of the samples, 11 females and 12 males. It had two bands, one corresponding to that observed in phenotype ESD FF and the other corresponding to that observed in phenotype ESD SS. There was no significant sex difference in the distribution of phenotypes. Esterase D bands specifically utilise 4-methyl umbelliferyl acetate as the substrate and are not stained by α- or β-napthyl acetate (Hopkinson *et al.*, 1973).

It is proposed that the esterase D phenotypes in *Trichogaster pectoralis* are controlled by an autosomal locus with two codominant alleles, ESD^F which gives rise to the fast band and ESD^S which gives rise to the slow band. Phenotype ESD FS is then due to heterozygosity. The allelic frequencies, assuming this hypothesis, were calculated by the method of gene counting. As the sample of adult fish typed was random, with 45 males and 40 females present, there was no reason to doubt the existence of Hardy-Weinberg equilibrium in this population. This was tested by the calculation of chi-square and it was found that the observed numbers did not differ significantly from the expected numbers (Table 1). This finding supported the hypothesis

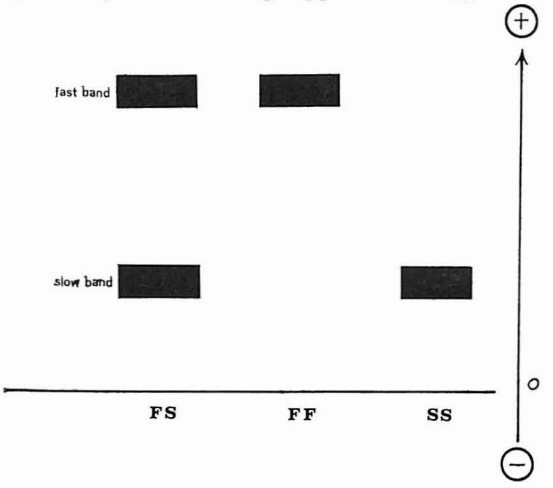

Figure 1. Diagrammatic representation of the three types of phenotypes of esterase D in Sepat Siam. O is the origin.

TABLE 1

Esterase D phenotypes in *Trichogaster pectoralis*.
The numbers within parentheses are the expected equilibrium numbers for each of the phenotypes.

	Phenotypes			Gene Frequencies	
Total	SS	FF	SF	ESD	ESD
85	60	2	23	0.841 ± 0.028	0.159 ± 0.028
	(60.15)	(2.14)	(22.71)	$\chi^2_1 = 0.0132$	P > 0.90

proposed for the inheritance of the esterase D phenotypes. In other vertebrates that have been examined and show variation in this enzyme, for example man and sheep, the genetic control is also that of an autosomal locus with two common co-dominant alleles. (Hopkinson *et al.*, 1973, McDermid *et al.*, 1975).

Five phenotypes were observed when both α- and β-napthyl acetate were present in the staining solution. These phenotypes observed for the general esterases are represented diagrammatically in Figure 2 and the number of individuals having each phenotype is given in Table 2. When only α-napthyl acetate was used as substrate, band 6 was not observed whereas when only β-napthyl acetate was the substrate, band 4 was absent. Hence band 4 is specifically stained for by α-napthyl acetate and band 6 by β-napthyl acetate while bands 1, 2, 3 and 5 can be stained for by both α- and β-napthyl acetate.

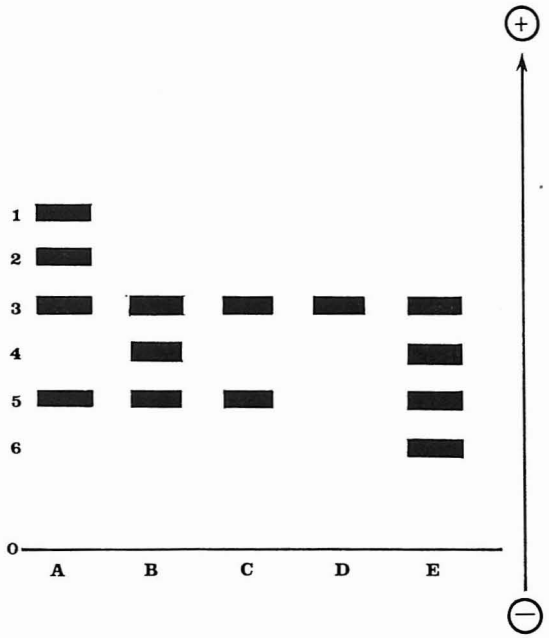

Figure 2. The phenotypes of liver general esterases when both the α- and β-napthyl acetate were used as substrates. Bands 1, 2, 3 and 5 were present when either α- or β-napthyl acetate alone was present as in (A). Band 4 was absent when β-napthyl acetate was used alone (C, D) and band 6 was absent when α-napthyl acetate was the only substrate (A, B, C, D). O is the origin.

Based on these observations and knowing the situation that exists in other species of fish (Kimura, 1978; Allendorf *et al.*, 1977; Kornfield *et al.*, 1978) it is proposed that four loci may be involved in controlling the liver general esterase types. The results, classified according to this hypothesis are presented in Table 3. Locus I is responsible for the presence of the most anodal bands 1, 2 and 3. These bands can use both α- and β-napthyl acetate as substrates. This locus has two codominant alleles, ES I^S being responsible for band 3 and ES I^F being responsible for band 1. The single female with bands 1, 2 and 3 can then be said to be a heterozygote and the middle band 2 is a hybrid band for a dimeric enzyme. The allelic frequencies were calculated by gene counting and Hardy-Weinberg equilibrium was tested for these data. The result showed that the observed numbers do not differ significantly from the expected numbers.

Locus II is responsible for the presence or absence of band 4 which only utilises α-napthyl acetate as substrate. Two alleles are present at this locus the dominant allele, ES II^+ is responsible for the presence of band 4 and the recessive allele, ES II^- causes its absence.

Locus III is responsible for the presence or absence of band 5 which utilises both α- and β-napthyl acetate as substrate. The presence of band 5 is due to the dominant allele, ES III^+ while its absence is due to homozygosity for the recessive allele, ES III^-.

Since band 6 (present in a single male specimen) is the only band which is specific for β-napthyl acetate, it is proposed that it is con-

trolled by a fourth locus. The rare dominant allele, ES IV$^+$, causes its presence whereas the common recessive allele, ES IV$^-$ causes its absence.

TABLE 2

The general esterase isozyme. This table shows the number of individuals having the different banding patterns when only α- or β-napthyl acetate or both were used as the substrate.

Substrate	Number of individuals	Presence of band number
α-napthyl acetate	1	1, 2, 3, 4, 5.
	52	3, 4, 5.
	37	3, 5.
	6	3.
	N = 96	
β-napthyl acetate	1	1, 2, 3, 5.
	1	3, 5, 6.
	88	3, 5.
	6	3.
	N = 96	
Both α-napthyl and β-napthyl acetate.	1	1, 2, 3, 4, 5.
	1	3, 4, 5, 6.
	88	3, 4, 5.
	6	3.
	N = 96	

Allelic frequencies for loci II, III and IV were calculated assuming Hardy-Weinberg equilibrium. Since loci II and III each had a substantial proportion of individuals with the presence and absence of their respective bands, a test was done to determine whether their observed combined phenotypes agree with those expected according to the hypothesis proposed for their genetic control. The expected phenotypic distribution is type 4, 5 = 49.68, type 4 = 3.31, type 5 = 40.32, null type (absence of bands 4 and 5 simultaneously) = 2.69. The observed numbers are 53, 0, 37 and 6 respectively. Since types 4 and null each had an expected value that is less than 5, they were combined so as to correct for continuity and gave an expected value of 6 for purposes of doing the chi-square test (Steward, 1976). The observed and expected numbers do not differ significantly from one another ($x_2^2 = 0.4946$, $P > 0.70$).

In animals, it is usual for the general esterases (that is esterases that use α- or β-napthyl acetate or both as substrates) from blood, saliva and body tissues to be controlled by several loci. This has been found to be so for humans (Coates *et al.*, 1975; Tan, 1976), farm animals, (McDermid *et al.*, 1975) and fishes, for example Japanese fresh water loach, *Cobitis delicata* (Kimura, 1978), Scandinavian fresh water trout, *Salmo trutta* (Allendorf *et al.*, 1977) and various species of the cichlid fishes of the Sea of Galilee (Kornfield *et al.*, 1978).

From this study we conclude that there is evidence for polymorphism in the liver esterases in the "Sepat Siam". Therefore they could be

TABLE 3

General esterase phenotypes and gene frequencies of *Trichogaster pectoralis* classified according to the proposed 4 loci mode of inheritance. Numbers within parentheses are the expected equilibrium numbers. + = presence, − = absence of band.

Locus I Band 1, 2, 3 Phenotypes			Locus II Band 4		Locus III Band 5		Locus IV Band 6	
FF (1)	SS (3)	FS (1, 2, 3)	+	−	+	−	+	−
0	95	1	53	43	90	6	1	95
(0.00)	(95.04)	(0.96)						
ES I^F = 0.005 ± 0.005			ES II$^+$ = 0.331 ± 0.034		ES III$^+$ = 0.750 ± 0.031		ES IV$^+$ = 0.005 ± 0.005	
ES I^S = 0.995 ± 0.005			ES II$^-$ = 0.669 ± 0.034		ES III$^-$ = 0.250 ± 0.031		ES IV$^-$ = 0.995 ± 0.005	
$\chi_1^2 = 0.0017$, $P > 0.95$								

useful as genetic markers in future studies of this economically important species of fish.

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