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ESTERASE ISOZYMES IN THE MOSQUITO *CULEX (MELANOCONION) ERRATICUS*

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ABSTRACT. Data obtained from cellulose acetate electrophoresis indicate that there are at least 5 separate loci for esterase (EST) activity in the Gorgas Memorial Laboratory colony of *Culex (Melanoconion) erraticus*. Locus EST-1 has a single band of activity which is not present in larvae. Locus EST-2 also has a single band which is present in larvae and pupae but is lost about 5 days after emergence. Locus EST-3 has three alleles, locus EST-4 two alleles, and locus EST-5 three alleles.

INTRODUCTION

In recent years there have been many reports of electrophoretic studies of esterase (EST) isozymes in mosquitoes. Townson (1971) described the genetics of certain EST isozymes in *Aedes aegypti*, and Saul et al. (1976) discussed EST variation in strains, of the same species. A total of 13 sites of EST activity was reported by Garnett and French (1971) in *Culex pipiens quinquefasciatus*, and de Stordeur (1976) reported ten EST isozymes in *Cx. pipiens pipiens*. Gargan (1977) studied the linkage relationships of two EST loci in *Cx. pipiens*. In a study on *Cx. tritaeniorhyncus*, Iqbal et al. (1973) reported a genetic analysis of an EST locus. A series of papers by Narang and Kitzmiller (1971 a, b; 1973 a, b) on *Anopheles punctipennis* reported a genetic analysis of multiple EST loci in natural populations. Green (1977) reported a sex-limited EST in *An. funestus*, and Miles (1978) reported a survey of enzyme variability in members of the *An. gambiae* group.

In most of these studies the authors reported multiple bands of EST activity in single mosquitoes, and they concluded that these bands represented the products of more than one locus. EST zymograms of *Cx. (Melanoconion) erraticus* Dyar and Knab also contain multiple bands per mosquito, and this study was undertaken

to determine if these bands represented the products of more than one locus.

MATERIAL AND METHODS

The mosquitoes used in this study were from the Gorgas Memorial Laboratory (GML) colony established from specimens collected near the Bayano study area, in the Republic of Panama. Electrophoresis was performed on larvae, pupae, and adults of both sexes, and was carried out on Titan III (Helena Laboratories) cellulose acetate membranes. *Cell buffer*: 0.1 M Trizma Base; 0.1 M Malcic acid adjusted to pH 7.2. *Membrane buffer*: 1 part cell buffer to 14 parts distilled water. *Reaction buffer*: 0.1 M NaH_2PO_4; 0.1 M Na_2HPO_4; adjusted to pH 6.3. *Esterase stain*: 150 mg α-naphthyl acetate and 50 mg β-naphthly acetate were dissolved in 10 ml of 50% aqueous acetone. Fifty mg Fast blue RR salt were dissolved in 30 ml reaction buffer. These two were combined with 30 ml 2% Noble Agar (50°C), 10 ml portions were placed in 100 × 15 mm petri dishes, and were stored at 3°C until needed.

Specimens were run 20 min. at 180 volts. The zymograms were incubated about 30 min. or until the bands were dark enough to observe. The full details of the cellulose acetate techniques were reported by Kreutzer et al. (1977).

Single pair matings were made between adults which emerged in individual vials. A male and female were placed in a 1-gal ice cream container. After 7 days the male was sacrificed and stored for later study. The eggs were deposited about 3 days after blood meal ingestion, and the female was then sacrificed and stored for later study. These mosquitoes were stored in a Revco® at −70°C.

Specimens for the equilibrium tests were taken from a cage which contained several hundred adults.

RESULTS AND DISCUSSION

A total of 8 active anodally migrating EST isozymes and 2 different nulls have been recovered from the GML colony of *Cx. erraticus* (Fig. 1). These isozymes have been assigned to 5 separate loci. No sex/phenotype relationships were observed; therefore, data from males and females are combined in Tables 1 and 2.

The fastest moving band, 1.00, first appears in the pupal stage and is observed in adult specimens older than 14 days. This band is designated locus EST-1, and is the reference band of the other bands produced from adults (Fig. 1). The band, 0.99, of locus EST-2 first appears in the larval stage, is present through the pupal stadium, and is lost about 5 days after emergence. Ved Brat and Whitt (1975) reported a similar situation with EST isozymes in *An. albimanus*. These differences during ontogeny indicate that the 1.00 and .99 isozymes are products of different loci. No

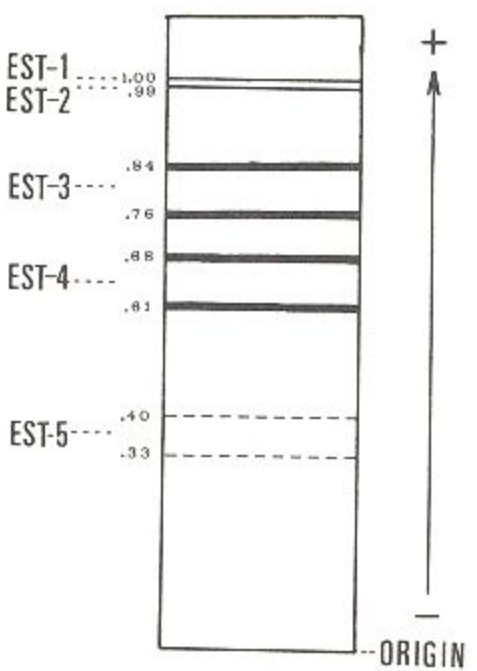

Figure 1. Diagramatic drawing of the active esterase isozymes in *Culex erraticus*. EST-1, etc. are esterase loci. The Rf values are to the left of the bands. Rf = migration distance of the band/migration distance of EST-1 band. Anode is at the top.

Table 1. Esterase phenotypes from single pair matings in *Culex erraticus*. EST-3, EST-4, EST-5.

Pair	Phenotypes				Parental genotypes[1]	Expected ratio				Chi-square	Probability[3]
EST-3	.84	.84/.76	.76	Null		.84	.84/.76	.76	Null		
1	—	—	—	43	N/N x N/N	0	0	0	1		
2	—	—	13	26	N/N x .76/N[2]	0	0	1	1	3.69*	>.05
3	32	—	—	9	.84/N x .84/N	3	0	0	1	0.07*	>.70
4	—	—	—	52	N/N x N/N	0	0	0	1		
5	—	5	21	—	.84/.76 x .76/.76[2]	0	1	1	0	8.65*	>.001
6	—	—	47	—	.76/.76[2] x .76/.76[2]	0	0	1	0		
7	—	—	10	19	.76/N[2] x N/N	0	0	1	1	2.21*	>.10
EST-4	.68	.68/.61		.61		.68	.68/.61		.61		
1	13	18		12	.68/.61 x .68/.61	1	2		1	1.19	>.50
2	23	16		—	.68/.61 x .68/.68	1	1		0	0.92*	>.30
3	41	—		—	.68/.68 x .68/.68	1	0		0		
4	52	—		—	.68/.68 x .68/.68	1	0		0		
5	—	26		—	.61/.61 x .68/.68	0	1		0		
6	47	—		—	.68/.68 x .68/.68	1	0		0		
7	—	12		17	.68/.61 x .61/.61	0	1		1	0.55*	>.30
EST-5	.40	.40/.33	.33	Null		.40	.40/.33	.33	Null		
1	43	—	—	—	.40/.40[2] x .40/.40[2]	1	0	0	0		
2	8	20	11	—	.40/.33 x .40/.33	1	2	1	0	0.48	>.70
3	24	—	—	17	.40/N[2] x N/N	1	0	0	1	0.88*	>.30
4	35	—	—	17	.40/N[2] x .40/N[2]	3	0	0	1	1.26*	>.20
5	—	—	—	26	N/N x N/N	0	0	0	1		
6	47	—	—	—	.40/.40[2] x .40/.40[2]	1	0	0	0		
7	29	—	—	—	.40/.40[2] x .40/.40[2]	1	0	0	0		

[1] Parental genotypes were determined by electrophoresis.
[2] These genotypes are assumed from the numbers obtained.
[3] Values obtained from abridged Table IV of Fisher and Yates.
* With Yates correction.

polymorphism was observed at either locus.

Preliminary study indicated that the other isozymes represented products of 3 loci. Tests were made to confirm this hypothesis. Individual pair matings were made, and the genotypes of the parents for the 3 loci were later determined by electrophoresis. The data from EST phenotypes among the progeny of each cross are recorded in Table 1. The probabilities show that the phenotypes of the progeny of most matings are consistent with the expected monohybrid frequencies for each locus; therefore the data support the hypothesis that 3 separate loci are involved.

The laboratory colony is a large mendelian population in equilibrium; then if these are separate loci the genotypic, and hence phenotypic, frequencies of the proposed alleles obtained from any large sample should be the same. Allele frequencies for each locus were determined from a control sample and equilibrium tests were made of the sample. In each case the control group was in equilibrium (Table 2). Additional samples, referred to as "test runs," were made and expected phenotypic frequencies for these new samples were determined using the established control sample gene frequencies. The "test run" phenotypic frequencies and their appropriate expected frequencies were compared. The data presented in Table 2 support the conclusion that EST-3, EST-4, and EST-5 are separate loci; furthermore, the data suggest that there are two active and a null allele at EST-3, two

Table 2. Test for equilibrium of the EST-3, EST-4, and EST-5 loci in the GML colony of *Culex erraticus*.

Locus	Test series	Genotype-phenotype	Theoretical frequency	Observed	Expected	
EST-3	Control run	.84/.84, .84/N	p^2+2pr	34	31.9	Frequency of .84=p=.1180[1]
		.84/.76	2pq	17	21.0	Frequency of .76=q=.3677
		.76/.76, .76/N	q^2+2qr	121	118.0	Frequency of Null =r=.5143
		N/N	r^2	59	59.0	χ^{2*}=0.71 p**>.30
	Test run	Same as	Same as	24	22.5	χ^{2*}=1.78 p**>.10
	using control	Est-3	EST-	9	14.4	
	run EST-3	control	control	88	85.2	
	allele frequencies	run	run	45	43.9	
	Test run	Same as	Same as	14	21.8	χ^{2*}=3.51 p**>.05
	using control	Est-3	EST-3	12	14.0	
	run EST-3	control	control	86	82.7	
	allele frequencies	run	run	49	42.6	
EST-4	Control run	.68/.68	s^2	133	136.0	Frequency of .68=s=.7707[2]
		.68/.61	2st	87	80.9	Frequency of .61=t=.2293
		.61/.61	t^2	9	12.0	χ^{2*}=0.96 p**>.30
	Test run	Same as	Same as	94	98.0	χ^{2*}=1.69 p**>.20
	using control	EST-4	EST-4	58	58.3	
	run EST-4	control run	control run	13	8.8	
	allele frequencies					
	Test run	Same as	Same as	85	91.5	χ^{2*}=1.30 p**>.20
	using control	EST-4	EST-4	57	54.4	
	run EST-4	control run	control run	12	8.8	
	allele frequencies					
EST-5	Control run	.40/.40, .40/N	u^2+2uw	135	133.7	Freuency of .40=u=.5452[3]
		.40/.33	2uv	23	25.0	Frequency of .33=v=.1147
		.33/.33, .33/N	v^2+2vw	20	18.2	Frequency of Null=w=.3401
		N/N	w^2	22	23.0	χ^{2*}=.19 p**>.50
	Test run	Same as	Same as	79	94.2	χ^{2*}=4.38 p**=.04
	using control	EST-5	EST-5	21	17.6	
	run EST-5	control	control	19	14.7	
	allele frequencies	run	run	20	16.3	

Table 2. Continued.

Locus	Test series	Genotype-phenotype	Theoretical frequency	Observed	Expected	
	Test run using control run EST-5 allele frequencies	Same as EST-5 control run	Same as EST-5 control run	114 28 22 19	122.3 22.9 17.7 21.2	$\chi^{2*}=2.37$ $p^{**}>.10$

[1] Calculation of allele frequencies with Null: Null=(Null phenotypes/n)$^{1/2}$, active allele=1-(individuals not having the allele/n)$^{1/2}$, n=total individuals. The frequencies were corrected according to Bernstein (in Li, 1954).

[2] s=proportion $s^2+1/2$ proportion st; t=proportion $t^2+1/2$ proportion st.

[3] Values obtained from abridged Table IV of Fisher and Yates.

* With Yates correction.

** Degrees of freedom for EST-3 and EST-5=4 phenotypes−3 alleles=1.
Degrees of freedom for EST-4=3 phenotypes−2 alleles=1.

active alleles at EST-4, and two active and a null allele at EST-5.

A typical EST zymogram is presented in Figure 2 with data from 8 different adults. In each specimen the band at locus EST-1 is present; however, no band is shown at EST-2 because these mosquitoes were run at least 8 days after emergence. Samples 1, 7, and 8 are homozygous null for locus EST-3; 2, 4, 5, and 6 have the .76 active allele; and specimen 3 has the .84 active allele. All samples have the .68 allele and 3, 4, and 6 are heterozygotes (.68 and .61) for locus EST-4. Individuals 3, 4, 6, 7, and 8 have the .41 allele; 1 and 5 the .33 allele; and specimen 2 is homozygous null for locus EST-5. Some of the specimens in the figure, especially evident at the EST-5 locus, show a very light lead band. This "extra" band is occasionally seen, and is probably an artifact of the zymogram preparation. The active alleles at loci EST-3, EST-4, and EST-5 are codominant or in the heterozygote for the active alleles there are 2 separate bands. Specimens with .84 and .76 bands, .68 and .61 bands, and .41 and .33 bands have been observed. Null is completely recessive to the active alleles. It is not possible to identify readily a null/active allele heterozygote.

Certain *Melanoconion* species have been involved with disease transmission, but many of these species cannot be easily identified with usual taxonomic char-

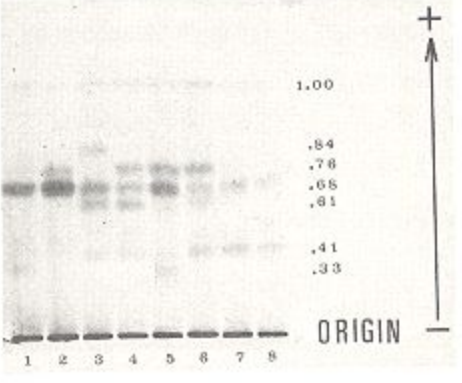

Figure 2. Zymogram of eight adult mosquitoes. See text for further explanation.

acters. Studies are under way to determine if EST polymorphism can be used to aid in the identification of the species within this subgenus.

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