GENETIC STUDIES ON TWO CARBOXYLESTERASE LOCI IN *AEDES ALBOPICTUS*

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ABSTRACT. Two esterase loci, *Est-4* and *Est-5*, in *Aedes albopictus* encode carboxylesterases in 4th-instar larvae, pupae and adults. The electrophoretic bands migrate on agar gels toward the most anodal side, those of *Est-5* followed by those of *Est-4*. Linkage studies on the two loci revealed that they were arranged on linkage group 2 in the following order: *Est-4*–(0.9 ± 0.7 to 4.3 ± 1.4 map units)–*p*(pigmented pupa)–(2 map units, as previously determined)–*Wb*(White-body)–(18.2 ± 2.6 to 21.0 ± 1.8 map units)–*Idh-2*(isocitrate dehydrogenase-2)–(13 map units, as previously determined)—*α-Gpdh*(α-glycerophosphate dehydrogenase)—(9.0 ± 1.8 to 19.9 ± 3.1 map units)–*Est-5*. The esterase loci were compared with those reported in other *Aedes* species with respect to their linkage homology.

INTRODUCTION

Comparative studies of genetic linkage maps among species or genera may give evolutionary information on the chromosomal divergences in the organisms. Foster et al. (1981) compared the linkage relationships of biochemical and morphological mutant loci among three dipteran species, *Lucilia cuprina* (Wiedemann), *Musca domestica* Linn., and *Drosophila melanogaster* (Meigen). They suggested that the major linkage groups have remained largely intact during the evolution of these Diptera. Munstermann and Craig (1979), who examined the linkage groups mapped with enzyme loci in *Aedes aegypti* (Linn.), *Culex pipiens* Linn., and *Cx. tritaeniorhynchus* Giles, indicated the occurrence of several chromosomal rearrangements in the three species. Such comparative genetics has extended to three or four more species of the *Aedes* mosquitoes (Pashley and Rai 1983). These investigations have provided a remarkable knowledge on chromosomal relationships among the *Aedes* species, but much more work along this line still remains to be done.

The high activity of esterases, hydrolyzing naphthylacetate substrates, is involved in resistance to organophosphate insecticides in the *Culex* mosquitoes (Yasutomi 1970, Georghiou and Pasteur 1978, 1980; Pasteur et al. 1981, Maruyama et al. 1984). Increased degradation of malathion by carboxylesterase has also been found in malathion-resistant *Cx. tarsalis* Coquillett (Matsumura and Brown 1961, 1963; Bigley and Plapp 1962). In the *Cx. pipiens* complex the *Est-3* and *-2* loci encoding esterases A′ and B, respectively, have been located on linkage group 3. The two loci are closely (only 0.67 map units apart) linked to each other (Pasteur et al. 1981).

This paper reports on the linkage relationship of two esterase loci encoding carboxylesterases (E.C. 3.1.1.1) in *Ae.* (*Stegomyia*) *albopictus* (Skuse).

MATERIALS AND METHODS

All strains or lines of the mosquitoes employed here were derived (or selected) from the Okinawa (Japan) strain, *p*(pigmented pupa), and *Wb*(White-body) mutant strains. Details of mosquito rearing and crossing experiments were described in a previous study (Tadano et al. 1980). In backcrosses, each egg batch was hatched in a single plastic box and separately reared as a family. Each family was scored for the phenotypes studied. Backcross data pooled from all the families were tested by chi-square at the 5% level for an expected 1:1 ratio of allelic segregation. Chi-square tests were applied also to the examination of genetic linkage at the 1% level.

Electrophoresis of esterases, α-glycerophosphate dehydrogenase, and isocitrate dehydrogenase was carried out by use of agar gels (Tadano 1982, 1984a). Characterization of esterases was made by using 10^{-4} M paraoxon (diethyl-p-nitrophenyl phosphate) and 10^{-2} M eserine sulfate acetone solutions (Tadano 1982).

RESULTS AND DISCUSSION

In a preliminary survey, the electrophoretic pattern of esterases were compared in several laboratory strains. Five zones of the esterase activity were observed on agar gels. Six esterase zones in *Ae. aegypti* were reported by Trebatoski and Craig (1969), Saul et al. (1976), Munstermann (1979)[1] and Field and Hitchen (1981). Both larval and pupal homogenates of *Ae. albopictus*, in general, gave more dense bands than the adults on every activity zone. This was true also for most of the esterase isozymes of *Ae.*

[1] Munstermann, L. E. 1979. Isozymes of *Aedes aegypti*. Phenotypes, linkage and use in the genetic analysis of sympatric subspecies populations in East Africa. Ph.D. thesis, University of Notre Dame. 176 pp.

aegypti electrophoresed on polyacrylamide gels (Saul et al. 1976).

The five zones were designated *Est-1* to *Est-5* in order of increasing mobilities (Fig. 1). Neither the 4th-instar larvae, pupae nor adults revealed clear, dense bands of *Est-1* and *-2* under electrophoretic conditions used. In the preliminary survey four or five alleles were detected at the *Est-5* locus, whereas three alleles plus a null allele were observed at *Est-4* with the null allele being most frequently detected in some strains. Since esterase activities of both *Est-4* and *-5* were inhibited by 10^{-4} M paraoxon but not affected at all by either 10^{-4} or 10^{-2} M eserine

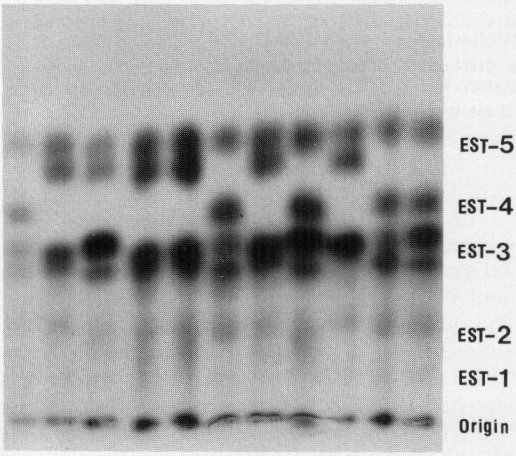

Fig. 1. Electrophoretic patterns of esterases in *Aedes albopictus*. The anodal side is at the top of this figure.

Table 1. A protocol of eight backcrosses of *Aedes albopictus* undertaken throughout this study.*

A. $\frac{m}{m}\ \frac{p}{p}\ \frac{Est\text{-}5^F}{Est\text{-}5^F} \times \frac{m}{M}\ \frac{+}{p}\ \frac{Est\text{-}5^S}{Est\text{-}5^F}$

B. $\frac{m}{m}\ \frac{Wb}{+}\ \frac{Est\text{-}5^F}{Est\text{-}5^S}\ \frac{Idh\text{-}2^I}{Idh\text{-}2^S} \times \frac{m}{M}\ \frac{+}{+}\ \frac{Est\text{-}5^S}{Est\text{-}5^S}\ \frac{Idh\text{-}2^S}{Idh\text{-}2^S}$

C. $\frac{m}{m}\ \frac{+}{+}\ \frac{Est\text{-}5^S}{Est\text{-}5^S}\ \frac{Idh\text{-}2^S}{Idh\text{-}2^S} \times \frac{m}{M}\ \frac{Wb}{+}\ \frac{Est\text{-}5^F}{Est\text{-}5^S}\ \frac{Idh\text{-}2^I}{Idh\text{-}2^S}$

D. $\frac{m}{m}\ \frac{+}{p}\ \frac{Est\text{-}5^S}{Est\text{-}5^F}\ \frac{Gpdh^F}{Gpdh^S} \times \frac{m}{M}\ \frac{p}{p}\ \frac{Est\text{-}5^F}{Est\text{-}5^F}\ \frac{Gpdh^S}{Gpdh^S}$

E. $\frac{m}{m}\ \frac{p}{p}\ \frac{Est\text{-}5^F}{Est\text{-}5^F}\ \frac{Gpdh^S}{Gpdh^S} \times \frac{m}{M}\ \frac{+}{p}\ \frac{Est\text{-}5^S}{Est\text{-}5^F}\ \frac{Gpdh^F}{Gpdh^S}$

F. $\frac{m}{m}\ \frac{Est\text{-}5^S}{Est\text{-}5^S}\ \frac{Est\text{-}4^N}{Est\text{-}4^N} \times \frac{m}{M}\ \frac{Est\text{-}5^S}{Est\text{-}5^F}\ \frac{Est\text{-}4^N}{Est\text{-}4^F}$

G. $\frac{m}{m}\ \frac{p}{+}\ \frac{Est\text{-}5^F}{Est\text{-}5^S}\ \frac{Est\text{-}4^N}{Est\text{-}4^I} \times \frac{m}{M}\ \frac{p}{p}\ \frac{Est\text{-}5^F}{Est\text{-}5^F}\ \frac{Est\text{-}4^N}{Est\text{-}4^N}$

H. $\frac{m}{m}\ \frac{+}{p}\ \frac{Est\text{-}5^S}{Est\text{-}5^F}\ \frac{Est\text{-}4^I}{Est\text{-}4^N} \times \frac{m}{M}\ \frac{p}{p}\ \frac{Est\text{-}5^F}{Est\text{-}5^F}\ \frac{Est\text{-}4^N}{Est\text{-}4^N}$

* The genotypes, m/m and M/m, in this table indicate female and male parents, respectively.

Table 2. Phenotypic scores of the backcross offspring obtained from crosses A through E.

Phenotype	Cross	A	Phenotype	Crosses	B	C	Phenotype	Crosses	D	E
Female	$+\ Est\text{-}5^{F/F}$	15	$+\ Est\text{-}5^{S/S}$	$Idh\text{-}2^{S/S}$	150	52	$+\ Est\text{-}5^{F/F}$	$Gpdh^{S/S}$	43	29
(m/m)	$+\ Est\text{-}5^{F/S}$	28	$+\ Est\text{-}5^{S/S}$	$Idh\text{-}2^{I/S}$	4	1	$+\ Est\text{-}5^{F/F}$	$Gpdh^{F/S}$	9	10
	$p\ Est\text{-}5^{F/F}$	21	$+\ Est\text{-}5^{F/S}$	$Idh\text{-}2^{S/S}$	57	34	$+\ Est\text{-}5^{F/S}$	$Gpdh^{S/S}$	2	4
	$p\ Est\text{-}5^{F/S}$	16	$+\ Est\text{-}5^{F/S}$	$Idh\text{-}2^{I/S}$	61	18	$+\ Est\text{-}5^{F/S}$	$Gpdh^{F/S}$	73	48
Male	$+\ Est\text{-}5^{F/F}$	12	$Wb\ Est\text{-}5^{S/S}$	$Idh\text{-}2^{S/S}$	44	18	$p\ Est\text{-}5^{F/F}$	$Gpdh^{S/S}$	70	31
(M/m)	$+\ Est\text{-}5^{F/S}$	17	$Wb\ Est\text{-}5^{S/S}$	$Idh\text{-}2^{I/S}$	67	29	$p\ Est\text{-}5^{F/F}$	$Gpdh^{F/S}$	0	4
	$p\ Est\text{-}5^{F/F}$	14	$Wb\ Est\text{-}5^{F/S}$	$Idh\text{-}2^{S/S}$	3	3	$p\ Est\text{-}5^{F/S}$	$Gpdh^{S/S}$	12	15
	$p\ Est\text{-}5^{F/S}$	12	$Wb\ Est\text{-}5^{F/S}$	$Idh\text{-}2^{I/S}$	147	65	$p\ Est\text{-}5^{F/S}$	$Gpdh^{F/S}$	47	25
Total		135	Total		533	220	Total		256	166
Families pooled		2	Families pooled		4	2	Families pooled		3	2

Table 3. Phenotypic scores of the backcross offspring obtained from crosses F, G, and H.

Phenotype	Cross	F	Phenotype	Crosses	G	H
$Est\text{-}5^{S/S}$	$Est\text{-}4^{N/N}$	63	$+\ Est\text{-}5^{F/F}$	$Est\text{-}4^{N/N}$	3	1
$Est\text{-}5^{S/S}$	$Est\text{-}4^{F/N}$	72	$+\ Est\text{-}5^{F/F}$	$Est\text{-}4^{I/N}$	39	33
$Est\text{-}5^{F/S}$	$Est\text{-}4^{N/N}$	55	$+\ Est\text{-}5^{F/S}$	$Est\text{-}4^{N/N}$	3	1
$Est\text{-}5^{F/S}$	$Est\text{-}4^{F/N}$	109	$+\ Est\text{-}5^{F/S}$	$Est\text{-}4^{I/N}$	66	80
			$p\ Est\text{-}5^{F/F}$	$Est\text{-}4^{N/N}$	63	70
			$p\ Est\text{-}5^{F/F}$	$Est\text{-}4^{I/N}$	3	0
			$p\ Est\text{-}5^{F/S}$	$Est\text{-}4^{N/N}$	33	30
			$p\ Est\text{-}5^{F/S}$	$Est\text{-}4^{I/N}$	0	0
Total		299	Total		210	215
Families pooled		4	Families pooled		3	3

sulfate, both esterases were concluded to be carboxylesterases.

Appropriate lines, which were used for eight backcrosses (Table 1) to map the *Est-4* and *-5* loci, had been selected from mutant and wild-type strains. Females have the *m/m* and males the *M/m* sex genotypes on linkage group 1 as in other culicine mosquitoes studied (Bat-Miriam and Craig 1966, Tadano et al. 1980). Linkage group 2 contains: *p*(pigmented pupa)–(2 map units)–*Wb*(White-body)–(7.5 to 17.8 map units)–*Idh-2*(isocitrate dehydrogenase-2)–(13.1 map units)–*α-Gpdh*(α-glycerophosphate dehydrogenase) (Tadano 1984a). In the above backcrosses, these morphological and enzyme loci were used with appropriate combinations of the following alleles: *I*(intermediate) and *S*(slow) alleles of *Idh-2*; *F*(fast) and *S* alleles of *α-Gpdh*; *F* and *S* alleles of *Est-5*; *F*, *I*, and *N* (null) alleles of *Est-4*. *Idh-2* and *α-Gpdh* produced dimeric enzymes as in an earlier study (Tadano 1984a), while *Est-5* and *-4* produced monomeric ones and the null allele of *Est-4* behaved as a complete recessive.

The offspring produced by cross A (Table 2) revealed the expected 1:1 ratio of allelic segregations at the *p* and *Est-5* loci ($P > 0.05$), but more female offspring were produced than males ($\chi^2 = 4.62$, $0.05 > P > 0.02$). Chi-square tests indicated linkage of *Est-5* with *p* ($P < 0.01$) but not with sex locus (*M/m*), the recombination units between *Est-5* and *p* being 40.7 ± 4.2 (Table 4). Since *Est-5* could be located on linkage group 2, four more crosses, B through E, were made for more precise mapping. In the four crosses, all alleles segregated at the 1:1 ratio. Recombination units among these loci are tabulated in Table 4. Crosses B and C indicated the gene arrangement of *Wb*–(18.2 ± 2.6 to 21.0 ± 1.8 map units)–*Idh-2*–(24.6 ± 1.8 to 30.5 ± 3.1 map units)–*Est-5*. Moreover, D and E gave the arrangement of *p*–(35.9 ± 3.0 to 37.4 ± 3.7)–*α-Gpdh*–(9.0 ± 1.8 to 19.9 ± 3.1)–*Est-5*. Thus, the gene order on linkage group 2 must be: *p–Wb–Idh-2–α-Gpdh–Est-5*.

Although variations in recombination units between sexes have been often documented in *Ae. aegypti*, *Cx. pipiens* and *Cx. tritaeniorhynchus* (Macdonald and Sheppard 1965, Craig and Hickey 1967, McClelland and Smithson 1968, Barr 1969, Baker and Sakai 1972, McClelland 1978), the sex-related differences in recombination units were not consistent in the present results (Table 4). This is contrary to a previous study (Tadano 1984a), for unknown reasons, which reported that female heterozygotes of *Ae. albopictus* exhibited more recombination than the males.

A first attempt to locate the *Est-4* locus was made by cross F (Table 3). This provided evi-

Table 4. Recombination units (±S.E.) calculated from data of eight crosses A through H.

Between	A (Male)*	B (Female)	C (Male)	D (Female)	E (Male)	F (Male)	G (Female)	H (Female)
p — Est-5	40.7 ± 4.2			47.7 ± 3.1	47.6 ± 3.9		35.7 ± 3.3	29.8 ± 3.1
p — Est-4							4.3 ± 1.4	0.9 ± 0.7
p — α-Gpdh				35.9 ± 3.0	37.4 ± 3.7			
α-Gpdh — Est-5				9.0 ± 1.8	19.9 ± 3.1			
Wb — Est-5		43.0 ± 2.1	45.0 ± 3.4					
Idh-2 — Est-5		24.6 ± 1.8	30.5 ± 3.1					
Wb — Idh-2		21.0 ± 1.8	18.2 ± 2.6					
Est-5 — Est-4						42.5 ± 2.9	37.1 ± 3.3	29.8 ± 3.1

* Sexes in these parentheses indicate parents heterozygous for the alleles involved in backcrosses.

dence for linkage of *Est-4* with *Est-5* ($\chi^2 = 6.77$, $P < 0.01$) and gave recombination units of 42.5 ± 2.9 between the two loci (Table 4). In this cross more $Est\text{-}4^{F/N}$ individuals were yielded than the $Est\text{-}4^{N/N}$ ($\chi^2 = 13.38$, $P \ll 0.01$). The subsequent G and H crosses, involving *p* and *Est-5*, placed *Est-4* more precisely on linkage group 2. In both crosses all alleles segregated at the 1:1 ratio ($P > 0.05$). Cross H gave equal recombination units (29.8 ± 3.1) for *Est-5–Est-4* as well as for *Est-5–p*, and only 0.9 ± 0.7 units for *Est-4–p*. Cross G indicated that the loci are arranged in the order of *Est-4*–(4.3 ± 1.4)–*p*–(35.7 ± 3.3)–*Est-5*, with the distance between *Est-4* and *Est-5* being the farthest (37.1 ± 3.3). These results here suggest that linkage group 2 in this species comprises a gene order of *Est-4–p–Wb–Idh-2–α-Gpdh–Est-5* with a total length of over 30 map units.

The esterase loci thus far mapped in the *Aedes* species are: *Est-4* (linkage group 1, Marvdashti 1985) and *Est-6* (linkage group 2, e.g., Munstermann and Craig 1979) in *Ae. aegypti*; *Est-5* (linkage group 2) in the *Ae. triseriatus* group (Munstermann et al. 1982); *Est-6* (linkage group 2) in the *Ae. scutellaris* group (Pashley and Rai 1983); and *Est-2* and *-3* (both on linkage group 1) in *Ae. togoi* (Tadano 1984b). Determination of homologies among these loci is difficult because of diverse properties of esterase isozymes. For example, *Est-6* of *Ae. aegypti* encodes acetylesterase particularly in the adult stage (Saul et al. 1976), while *Est-2* and *-3* of *Ae. togoi*, as well as *Est-4* and *-5* of *Ae. albopictus* encode carboxylesterases. However, it is of interest that *Est-5* of both *Ae. albopictus* and the *Ae. triseriatus* group, and also *Est-6* of both *Ae. aegypti* and the *Ae. scutellaris* group are all located on very similar regions of the respective linkage group 2.

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