THE GENETICS OF AN ESTERASE IN *CULEX TRITAENIORHYNCHUS*

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ABSTRACT. The genetic analysis of an esterase polymorphism at the Est-4 locus in *Culex tritaeniorhynchus* is presented. A series of five co-dominant alleles have been uncovered in a survey of laboratory strains. Linkage analysis suggests that the Est-4 locus is located on one of the autosomes.

Multiple molecular forms of esterases have been studied in several mosquito species (Townson, 1969; Trebatoski and Craig, 1969; Bianchi and Rinaldi, 1970; Narang and Kitzmiller, 1971a, 1971b; Garnett and French, 1971). This interest in the investigation of esterase polymorphisms reflects the need felt by many workers for useful genetic markers. Moreover, enzyme polymorphisms have been used to study natural populations of mosquitoes (Coluzzi and Bullini, 1971).

A survey of strains of *Culex tritaeniorhynchus* maintained in our laboratory has revealed five areas of esterase activity which were designated areas 1, 2, 3, 4 and 5 in ascending order toward the anode. The resolution of areas 1, 2, 3 and 5 was not always clear. On the other hand, area 4 showed consistently clear resolution, and in addition, a high degree of variability in the mobility of the bands. Therefore, the genetics of the zones of the area, herein called Est-4 was investigated. Within the Est-4 area, five major bands of esterase activity were found which were designated A, B, C, D and E in ascending order toward the anode. Although five zones of activity have been uncovered among the laboratory stocks, individual mosquitoes had only 1 or 2 zones of esterase activity in the Est-4 area. This paper reports the results of experiments aimed at elucidating the genetics of two of these bands (Est-4C and Est-4D) in this mosquito species.

MATERIALS AND METHODS. The following stocks of *Culex tritaeniorhynchus* were used in this study:

A. ***Esterase — 4 C (Est-4C):*** This strain was started from individuals collected in Khulna, in November, 1967. It is true-breeding for Est-4C.

B. ***Esterase — 4 D (Est-4D):*** This strain originated from females collected at the Ravi River, Lahore, Pakistan, in October, 1966. It breeds true for Est-4D.

Crossing techniques and rearing procedures are similar to those reported previously (Rabbani and Baker, 1970; Baker and Sakai, 1972). Individual egg rafts (single families) were isolated into vials, and after hatching had occurred, the number of hatched and unhatched eggs were recorded for each raft. Each family was reared individually in a 1-liter Erlenmeyer flask. The larvae were fed edible liver powder. The adults emerging daily were classified according to sex and tested for esterase or were frozen at −70° C for future testing.

For the electrophoretic separation of the esterases, each adult was macerated in 0.01 ml of tris-citrate buffer, pH 7.9. The crude extract was absorbed into a 3 x 6 mm piece of Whatman #1 filter paper which was then inserted into a precut slot in the gel. Horizontal gel electrophoresis was carried out using an 11 percent concentration of starch (Connaught Laboratories) and a modification of a discontinuous system of buffers as described by Poulik (1957). Electrophoresis was carried out at 8V/cm for approximately 4 hours or until the brown borate zone had migrated approximately 7 cm from the sample slot. The zones of esterase activity were visualized by immersion of the sliced gel into a

solution consisting of phosphate buffer, pH 6.0, alpha-naphthyl acetate and fast blue RR salt (Beckman and Johnson, 1964).

In this paper the genes of the proposed genotype which appear above the line are derived from the mother and those below are of paternal origin. The statistical analysis follows that of Bailey (1961).

RESULTS AND DISCUSSION. Table 1 summarizes the results of crosses used to elucidate the genetics of Est-4. Crosses A and B represent crosses which were made between members within each true-breeding stock. Crosses C and D represent the reciprocal parental matings between the two stocks. Crosses E through H represent the backcrosses of heterozygous females to homozygous males of the two stocks. Crosses I through J are the backcrosses of heterozygous males to homozygous females. Table 2 presents the chi-square analysis for these crosses.

CROSSES WITHIN STOCK. Cross A is a single family (egg raft) randomly isolated from our true-breeding Est-4C stock and cross B is a family from our Est-4D stock. The results support our observations that both our stocks breed true for their respective esterase pattern (C/C and D/D respectively). Chi-squares for the 1:1 segregation between the sexes among the progeny in both crosses were not significant.

PARENTAL CROSSES. Crosses C and D represent reciprocal crosses between the two esterase stocks. All the progeny from both crosses were characterized by two zones of esterase activity (one in the C position and one in the D position, see Figure 1). This suggests that these esterases are co-dominant alleles as both parental phenotypes are observed in the hybrid progeny. The segregation for sex did not show significant departures from the expected for either cross.

BACKCROSSES TO HETEROZYGOUS FEMALES. In the backcrosses of heterozygous females to the two types of homozygous males, cross G showed a highly significant departure from the expected 1:1 ratio for sex. One of the reciprocal crosses to the D/D ♂ (cross E) also departed significantly from the 1:1 expected ratio of C/D: D/D. There was a large excess of C/D individuals. Cross F and the two reciprocal crosses to the C/C ♂ (crosses G and H) did not show any significant departure from the expected 1:1 for C/D:D/D and C/C:C/D respectively.

BACKCROSSES TO HETEROZYGOUS MALES. Cross J among the backcrosses to the heterozygous males showed a significant chi-square for the 1:1 segregation for sex. Both backcrosses to D/D females (crosses I and J) showed highly significant departures from the expected 1:1 ratio of C/D:D/D. It may be noted here that in three of the four crosses involving backcrosses to D/D individuals, there were significant departures from the expected 1:1 segregation of C/D:D/D. These three crosses were characterized by large excesses of C/D individuals, suggesting that under our experimental conditions the D/D individuals have a lowered viability. Beckman and Johnson (1964) and Garnett and French (1971) have also observed that certain homozygous combinations of esterase alleles appear to show lower viability in comparison to heterozygous combinations. Chi-squares for the C/C:C/D ratio in crosses K and L were not significant.

LINKAGE BETWEEN EST-4 AND SEX (M,M). Chi-squares to detect linkage between Est-4 and sex were calculated for

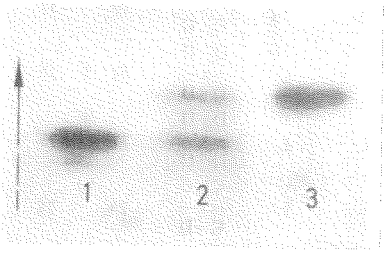

FIG. 1.—Photograph of a starch gel stained for esterase. 1, Est-4C/Est-4C; 2, Est-4C/Est-4D; 3, Est-4D/Est-4D. The arrow shows the direction of migration.

crosses E through L. The four crosses involving heterozygous females (E through H) did not show any significant chi-squares for linkage between the Est-4 locus and sex. This is expected as females of this species are the homogametic (*m*/*m*) sex. Linkage chi-squares for the crosses with the heterozygous males (J through L) also were not significant. This is evidence that Est-4 is not sex-linked. Support for this hypothesis comes from some preliminary experiments with the sex-linked mutant *golden* (Baker, 1968), in which no evidence of linkage could be found between golden and Est-4. Thus, it seems reasonable to conclude that the Est-4 locus is located on one of the autosomes. Experiments are now underway to assign this esterase locus to either chromosome 2 or 3.

OTHER ALLELES AT THE EST-4 LOCUS. A survey of the strains of *Culex tritaenio-*

TABLE 1.—Pooled results of crosses to elucidate the genetics of Est-4.

					Progeny phenotype					
	Parental genotype				♀			♂		
Cross	F*	♀ ♂	% hatch	% rearing	C/C	C/D	D/D	C/C	C/D	D/D
A	1	C C / C C	74	70	41	0	0	49	0	0
B	1	D D / D D	94	44	0	0	39	0	0	34
C	1	C D / C D	93	97	0	56	0	0	43	0
D	1	D C / D C	99	47	0	43	0	0	31	0
E	4	C D / D D	89	76	0	157	90	0	120	94
F	3	D D / C D	99	67	0	60	54	0	53	73
G	3	C C / D C	87	64	92	74	0	58	58	0
H	3	D C / C C	99	57	41	68	0	52	52	0
I	5	D C / D D	84	78	0	112	71	0	121	65
J	4	D D / D C	77	75	0	83	53	0	69	30
K	7	C C / C D	76	75	122	122	0	109	127	0
L	5	C D / C C	80	58	70	68	0	57	62	0

* Number of families examined.

TABLE 2.—Chi-square analysis of data presented in Table 1.

Cross	1:1 Segregation			Linkage
	m:M	C/D:D/D	C/C:C/D	Sex:Est-4
A	0.71	....	....	....
B	0.34	....	....	....
C	1.71	....	....	....
D	1.94	....	....	....
E	2.36	18.76**	....	3.65
F	0.60	0.82	....	2.82
G	8.86**	....	1.15	1.15
H	0.12	....	3.42	3.42
I	0.02	25.50**	....	0.61
J	5.82*	20.26**	....	0.34
K	0.13	....	0.68	0.68
L	1.40	....	0.04	0.19

*P < 0.05.
**P < 0.01.

rhynchus maintained in our laboratory has uncovered three other alleles (A, B and E) at this locus. The mobilities of the alleles of Est-4 in ascending order toward the anode is as follows: A, B, C, D and E. No null allele for Est-4 has been found. Many of our strains, even those which have been maintained for nearly five years in laboratory culture, are still segregating for two and sometimes three alleles at the Est-4 locus. This is in agreement with our observations that some of the heterozygous combinations of the Est-4 alleles appear to show enhanced viabilities when compared to some of the homozygotes.

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